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PLANT AND SOIL

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International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases

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MARTINUS NIJHOFF THE HAGUE

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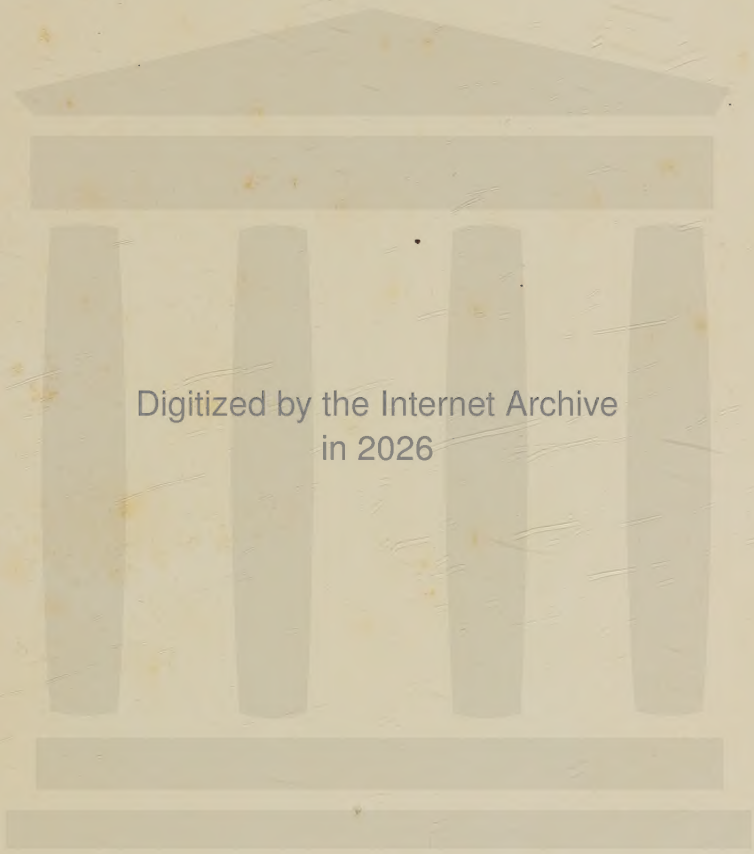
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PROFESSOR J. G. WOOD, D.SC., F.A.A.
1900-1959

Plant and Soil XV, no. 4 December 1961

PROFESSOR J. G. WOOD

Professor J. G. Wood died suddenly on 8th December 1959, to the grief of his many friends and serious loss to botanical work in Australia. He had been connected with Plant and Soil in the capacity of Consulting Editor since its first publication in1949.

Joseph Garnett Wood was born at Mitcham near Adelaide, South Australia, on 20th September 1900 and received his early education in that State. In 1922 he graduated with honours in Chemistry at the University of Adelaide and in the following year was appointed demonstrator in the Department of Botany. Research work on the ecology of the semi-arid parts of South Australia was just beginning and his chemical knowledge was of great importance as a collaborator. Working with Professor T. G. B. Osborn it was shown that the dominant Chenopodiaceous plants over vast areas were not halophytes in the ordinary sense of the term, as had been surmissed by W. A. Cannon. In 1925 the University of Adelaide received a gift of some 500 hectares for the establishment of a field laboratory at Koonamore, a very large sheep-grazing property in an area of some 20 cm average annual rainfall. Wood began work on the transpiration of arid plants and was able to show that water absorption was possible through the tomentose leaves of the dominant species of *Atriplex*, particularly *A. vesicaria*, a valuable sheep fodder plant.

In 1925 he was awarded an 1851 Research Scholarship and proceeded to Cambridge to work on Plant Physiology for the Ph.D. degree. On his return to Adelaide he rejoined the Botany Department, and when Professor Osborn was appointed to the Chair in Sydney (1928), Wood was made Lecturer-in-Charge. He was appointed Professor in 1935 and remained in that post for 25 years.

He twice served as Dean of the Faculty of Science and at the time of his death held the responsible positions of Chairman of the Research Executive Committee and of the Board of Research Studies in the University.

Outside the University he served on the Interim Council working to the formation of the National University at Canberra, and later on its Council; he had two periods of office on the executive council of the Commonwealth Scientific and Industrial Research Organization. He was elected Fellow of the Australian Academy shortly after its foundation in 1954. Wood twice visited the Netherlands during his study leaves and worked for some months at Leyden in 1938.

Wood's research work was in the fields of ecology, particularly of arid regions, and nutritional plant physiology. He collaborated with work at the Koonamore Vegetation Reserve from its beginning and later directed it. He had a wide knowledge of the vegetation of South Australia and in 1937 published a short book on it. Though he accepted the concept of succession he was critical of its application to many phases of Australian vegetation. His paper with Professor R. N. Crocker (one of his former students) on "Historical influences on the development of South Australian vegetation communities and their bearing on concepts and classification in ecology" (Trans. Roy. Soc. S. Australia Vol. LXXI, 1947) is important.

In Plant Physiology Wood collaborated with Dr. A. K. Petrie in a series of papers on the metabolism of nitrogen and sulphur compounds. Later he became interested in trace-element work, so important to South Australia. In many of his papers Wood was associated with a long series of graduate students, many of whom now hold responsible positions throughout Australia. Not the least important of Wood's many services to Science in Australia was his work on the Editorial Board and later Chairmanship of the Board of Standards of the Australian Journals of Scientific Research founded by C.S.I.R.O. and the Australian Academy.

It is not surprising that with his many outside activities Wood found relaxation in his home and garden. In the last few years he, and his wife, laid out and developed a very beautiful garden in

which he planted many exotics not usually grown in Adelaide but from similar climatic regions.

Biographical notices of Wood have appeared in various Australian journals, notably the Yearbook of the Australian Academy of Science 1960. That in Transactions of the Royal Society of South Australia LXXXIV, 1961, has a full bibliography of his published work.

Adelaide T. G. B. OSBORN

UBER DIE WIRKUNG VON CROTONYLIDENDIHARNSTOFF (CD-HARNSTOFF) ALS STICKSTOFFDUNGER

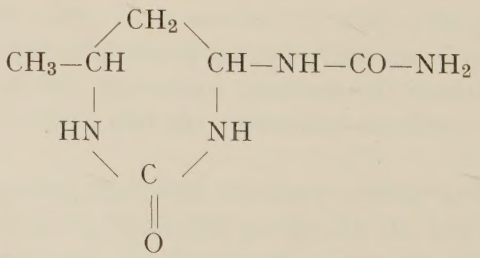
von J. JUNG

Aus der Landwirtschaftlichen Versuchsstation Limburgerhof

EINLEITUNG

Bereits seit längerer Zeit wird nach synthetischen Substanzen gesucht, die den Stickstoff langsam an die Pflanzen anliefern. Zu der in diesem Zusammenhang bisher am häufigsten untersuchten Gruppe von Stickstoffverbindungen zählen die Kondensationsprodukte von Harnstoff und Formaldehyd, die sog. „Ureaform"-Präparate [1 2 3 4 8]. Von Scheffer und Mitarbeitern [6 7] wurden neuerdings auch Kondensationsprodukte von Harnstoff und Acetaldehyd („Urea-Z") bearbeitet.

In der vorliegenden Arbeit soll über Ergebnisse mit einem Umsetzungsprodukt von Harnstoff und Crotonaldehyd = Crotonylidendiharnstoff (CD-Harnstoff) berichtet werden. Da es sich beim Crotonaldehyd um eine ungesättigte Verbindung handelt, ergeben sich hinsichtlich der Umsetzung mit Harnstoff grundsätzlich andere Reaktionsverhältnisse als bei gesättigten Aldehyden. Das erhaltene Kondensationsprodukt liegt – wie bereits Paquin [5] nachweisen konnte – aller Wahrscheinlichkeit nach als 2–Oxo–4–methyl–6–ureido–hexahydropyrimidin vor.



Zur Prüfung dieses Sachverhaltes haben W. Huber und H. Petersen („Ammoniak-Laboratorium" der BASF) versucht, etwa noch vorhandene Doppelbindungen nachzuweisen. Sämtliche Nachweisversuche, wie qualitativer Test mit $KMnO_4$, Hydrierjodzahl, Jodzahl nach mehreren Methoden, UR-Spektrum, fielen jedoch negativ aus. Da aus Harnstoff-Formaldehyd-Kondensaten beim Kochen mit Säuren quantitativ Formaldehyd abgespalten wird, wurde fernerhin versucht, in analoger Weise eine Crotonaldehyd-Abspaltung zu erreichen. Dies gelang nicht, was ebenfalls für die Ringstruktur spricht. Eine NH_2-Abspaltung nach van Slyke ergab das Vorliegen von zwei NH_2-Gruppen. Dies läßt sich ebenfalls nur mit der Ringstruktur vereinbaren.

Ein indirekter Beweis für die Ringstruktur des Crotonylidendiharnstoffs wurde auch durch die Synthese des Kondensationsproduktes von Crotonaldehyd und Dimethylharnstoff (1 : 2) erbracht. Diese Verbindung zeigt wesentlich andere Eigenschaften; sie reduziert $KMnO_4$ und ist in org. Lösungsmitteln löslich. Offenbar liegt hier wegen der Unmöglichkeit einer Ringbildung das offenkettige Derivat vor:

$$CH_3{-}CH = CH{-}CH < \begin{matrix} NH{-}CO{-}N(CH_3)_2 \\ NH{-}CO{-}N(CH_3)_2 \end{matrix}$$

VERSUCHSDURCHFÜHRUNG

Der Versuch wurde in Mitscherlich-Gefäßen auf einem Boden-Sandgemisch (lehmiger Sandboden und Quarzsand im Verhältnis 1 : 1) mit einem pH-Wert von 7.5 durchgeführt. Als Versuchspflanze wurde Weidelgras gewählt. Durch die Entnahme mehrerer Schnitte konnte die Anlieferung des Stickstoffs aus den verschiedenen Verbindungen während eines längeren Zeitraumes leicht verfolgt werden. Während der Periode März bis November konnten bei allen Reihen 4 Grasschnitte entnommen werden; mit einigen Reihen wurde der Versuch – während des Winters im Gewächshaus – bis Ende Mai des folgenden Jahres fortgesetzt.

In der Anordnung des Versuches liegt eine Unterteilung nach der Höhe der Wasserzufuhr vor: Im Versuchsglied *"ohne Auswaschung"* wurde der Boden auf 60% der maximalen Wasserkapazität gehalten, während im Versuchsglied *"mit Auswaschung"* 7 Tage vor dem ersten Schnitt einmal eine größere Wassermenge zugeführt wurde, die sich bei vorheriger Wassersättigung des Bodens auf 500 cm^3/Gefäß belief. Das Sickerwasser wurde aufgefangen und auf Stickstoff analysiert.

Neben Crotonylidendiharnstoff wurden auch noch folgende N-haltige

Substanzen bzw. Verbindungen bei einer Gabe von 1.0 g N/Gefäß gegen Ammonnitrat (als schnell wirkende N-Verbindung) vergleichend geprüft:

Geprüfte N-haltige Substanzen	N-Gehalt, %
1. Crotonylidendiharnstoff (CD-Harnstoff)	32.16
2. Handelsüblicher Ureaform-Dünger . . .	38.87
3. Acetylendiharnstoff	38.86
4. Oxamid	30.55
5. Glykokoll	18.44
6. Hippursäure	7.81
7. Casein	12.76

ERGEBNISSE

In Tabelle 1 wird die Ertragsbildung sowohl bei normaler Wasserversorgung, d.h. *ohne Auswaschung*, als auch bei hoher Wasserzufuhr, d.h. *mit Auswaschung*, aufgezeigt. Daraus ist zunächst für das Versuchsglied *ohne Auswaschung* zu entnehmen, daß die Ertragswirkung von Crotonylidendiharnstoff beim 1. und 2. Schnitt hinter jener der übrigen N-Verbindungen zurückbleibt, um dann bei den weiteren Schnitten durch eine deutliche Überlegenheit abgelöst zu werden.

Die Summe der Erträge vom 1. bis 4. Schnitt liegt beim Crotonylidendiharnstoff zwar niedriger als beim Ammonnitrat, deckt sich aber in etwa mit jener von Casein und Glykokoll und liegt wesentlich höher als die des Ureaformdüngers. Die Weiterführung des Versuches bis zum 6. Schnitt zeigte eine weitere Überlegenheit des Crotonylidendiharnstoffs gegenüber Ureaform und den Ausgleich in der Ertragsbildung zwischen Crotonylidendiharnstoff und Ammonnitrat. Insgesamt wurden somit in der Ammonnitrat-Reihe 50.7, in der Crotonylidendiharnstoff-Reihe 51.8 und in der Ureaform-Reihe 29.6 g Trockensubstanz/Gefäß geerntet; mit dem Unterschied, daß der Stickstoff aus dem Ammonnitrat schneller und der Stickstoff aus dem Crotonylidendiharnstoff langsamer angeliefert wurde.

Im Vergleichsglied mit hoher Wasserzufuhr, d.h. *mit Auswaschung*, ergibt sich eine nennenswerte Verschiebung in der Ertragsbildung zu Gunsten des Crotonylidendiharnstoffs, dadurch bedingt, daß durch die geringere Auswaschung dieser Verbindung den Pflanzen absolut höhere N-Mengen zur Verfügung standen.

Die im Sickerwasser festgestellten Stickstoffmengen waren folgende:

TABELLE 1

N-Verbindung	Ertragswirkung (g Trockensubstanz/Gefäß) der verschiedenen N-Verbindungen							
	1. Schnitt 16.6.58	2. Schnitt 16.7.58	3. Schnitt 10.9.58	4. Schnitt 26.11.58	5. Schnitt 3.3.59	6. Schnitt 26.5.59	1.-4. Schnitt	1.-6. Schnitt
(a) *ohne Auswaschung*								
ohne N	3.6	1.1	1.3	1.5	0.8	1.3	7.5	9.6
Ammonnitrat	11.5	25.2	8.4	2.6	1.4	1.6	47.7	50.7
Crotonylidendiharnstoff	5.7	13.5	14.8	6.0	6.5	5.3	40.0	51.8
Ureaform	9.0	7.5	6.1	2.5	2.0	2.5	25.1	29.6
Acetylendiharnstoff	10.8	24.9	9.1	3.2	—	—	48.0	—
Oxamid	9.1	24.9	9.6	2.2	—	—	45.8	—
Glykokoll	10.0	21.4	8.1	3.4	—	—	42.9	—
Hippursäure	9.1	17.2	6.3	2.3	—	—	34.9	—
Casein	10.1	22.2	7.6	2.8	—	—	42.7	—
(b) *mit Auswaschung*								
ohne N	3.3	1.1	1.9	1.6	1.1	1.5	7.9	10.5
Ammonnitrat	8.3	9.2	3.4	1.9	0.9	1.3	22.8	25.0
Crotonylidendiharnstoff	6.4	9.9	13.6	6.2	5.6	4.2	36.1	45.9
Ureaform	8.6	5.9	5.7	2.8	1.3	2.4	23.0	26.7
Acetylendiharnstoff	11.9	6.3	2.7	1.6	—	—	22.5	—
Oxamid	10.4	9.3	3.2	1.8	—	—	24.7	—
Glykokoll	10.1	6.5	3.4	2.3	—	—	22.3	—
Hippursäure	9.9	5.6	3.5	1.9	—	—	20.9	—
Casein	11.4	6.7	3.8	2.1	—	—	24.0	—

Versuchsreihe	Ausgewaschene N-Menge mg/Gefäß
Ohne N	46
Ammonnitrat	908
Crotonylidendiharnstoff	236
Ureaform	197
Acetylendiharnstoff	892
Oxamid	950
Glykokoll	665
Hippursäure	507
Casein	621

Diese Zahlen lassen erkennen, daß unter den verschiedenen Stickstoffverbindungen Crotonylidendiharnstoff und Ureaform die weitaus geringsten Auswaschungsverluste zeigen. Beim Crotonylidendiharnstoff ergibt sich demnach in der Ertragsbildung auch eine sehr starke Überlegenheit gegenüber den Verbindungen mit hoher N-Auswaschung. Dies trifft für Ureaform indessen nicht zu, da hier die an sich sehr niedrige N-Auswaschung durch die bereits in früheren Versuchen festgestellte niedrige N-Ausnutzung kompensiert wird. Die Ertragsunterschiede zwischen Ammonnitrat, Ureaform und Crotonylidendiharnstoff liegen nach dem 6. Schnitt bei 25 : 26 : 45 g Trockensubstanz/Gefäß.

Für eine vergleichende Beurteilung der geprüften Verbindungen ist neben der Ertragswirkung auch die Stickstoffaufnahme und die prozentuale Ausnutzung der zugeführten Stickstoffmenge von Interesse. Einschlägige Werte hierüber sind in Tabelle 2 angegeben.

Im zeitlichen Verlauf der Stickstoffaufnahme ist nach dem 2. Schnitt, außer bei Crotonylidendiharnstoff, eine weitgehende Erschöpfung der N-Anlieferung festzustellen. In der Reihe mit Crotonylidendiharnstoff ist nach relativ niedriger N-Anlieferung im Falle des 1. und 2. Schnittes eine überlegene Nachwirkung bis zum 6. Schnitt nachzuweisen. Dabei liegt die prozentuale N-Ausnutzung innerhalb eines Jahres mit *ca* 56 Prozent unter der des Ammonitrats mit *ca* 75 Prozent (oberirdische Pflanzenteile). Beim Ureaform wurde eine N-Ausnutzung von 34 Prozent gefunden.

Durch den Einfluß der N-Auswaschung, die unter Freilandverhältnissen stets zu berücksichtigen ist, ergibt sich eine nennenswerte Verschiebung hinsichtlich der prozentualen N-Ausnutzung zu Gunsten von Crotonylidendiharnstoff. Wie aus den Ergebnissen

TABELLE 2

N-Verbindungen	Stickstoffaufnahme (mg/Gefäß) aus den verschiedenen N-Verbindungen									
	1. Schnitt 16.6.58	2. Schnitt 16.7.58	3. Schnitt 10.9.58	4. Schnitt 26.11.58	5. Schnitt 3.3.59	6. Schnitt 26.5.59	1.-4. Schnitt	1.-6. Schnitt	Prozentuale Ausnutzung des zugeführten N 1.-4. Schnitt	Prozentuale Ausnutzung des zugeführten N 1.-6. Schnitt
(a) *ohne Auswaschung*										
ohne N	56	18	22	26	16	17	122	155	—	—
Ammonnitrat	534	587	81	33	21	19	1235	1275	74.20	74.67
Crotonylidendiharnstoff	153	305	238	125	121	51	821	993	46.80	55.87
Ureaform	345	129	83	42	38	30	599	667	31.80	34.13
Acetylendiharnstoff	521	615	84	38	—	—	1258	—	75.73	—
Oxamid	452	737	92	30	—	—	1311	—	79.27	—
Glykokoll	500	458	79	44	—	—	1081	—	63.93	—
Hippursäure	436	294	67	34	—	—	831	—	47.27	—
Casein	494	444	81	37	—	—	1056	—	62.27	—
(b) *mit Auswaschung*										
ohne N	58	17	26	29	21	19	130	170	—	—
Ammonnitrat	363	134	37	27	16	13	561	590	28.73	28.00
Crotonylidendiharnstoff	164	218	231	153	97	39	766	902	42.40	48.80
Ureaform	245	101	82	45	26	30	473	529	22.87	23.93
Acetylendiharnstoff	413	88	33	24	—	—	558	—	28.53	—
Oxamid	425	118	37	26	—	—	606	—	31.73	—
Glykokoll	387	92	42	35	—	—	556	—	28.40	—
Hippursäure	338	82	43	32	—	—	495	—	24.33	—
Casein	405	92	47	35	—	—	579	—	29.93	—

im Versuchsglied „mit Auswaschung" hervorgeht, liegt hier die prozentuale N-Ausnutzung für Ammonnitrat bei 28.0, für Ureaform bei 23.9 und für Crotonylidendiharnstoff bei 48.8 Prozent.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wird über die Eigenschaften des Crotonylidendiharnstoffs (CD-Harnstoff) als langsam wirkender Stickstoffdünger berichtet. Diese Verbindung unterscheidet sich chemisch von den in diesem Zusammenhang bisher am häufigsten untersuchten Kondensationsprodukten aus Harnstoff und Formaldehyd insbesondere durch ihre Ringstruktur. Daraus ergeben sich auch hinsichtlich der pflanzenphysiologischen Wirkung besondere Vorteile. Crotonylidendiharnstoff zeigt bei relativ hoher Ausnutzung seiner Stickstoffkomponente durch die Pflanzen eine ausgeprägt langsame Umsetzung und wird aus dem Boden nur sehr schwer ausgewaschen.

Eingegangen am 17. April 1961

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INFLUENCE OF SODIUM ON THE DEVELOPMENT OF THE FIBRO-VASCULAR BUNDLES IN HEMP PLANTS

by G. TREGGI

Istituto di Patologia Vegetale e Microbiologia Agraria e Tecnica della Università di Pisa

In the course of an investigation which has been carried on for some time at this Institute on the effect of sodium on the development of crop plants it has been established that considerable increases in the formation of fibro-vascular tissues result from increasing applications of this element.

This histological effect is particularly important in textile plants of which the fibre bundles are used. An investigation was consequently initiated in collaboration with the Plant Nutrition Research Laboratory at Wageningen on flax plants supplied with ascending doses of sodium adsorbed on dusarite as an artificial complex [2]. The successful results of this study led us to repeat it with another textile plant, hemp, to determine whether similar results would be obtained.

MATERIALS AND METHODS

The monoecious French variety of hemp, Fatza, was chosen for the experiment which was carried out at the Plant Nutrition Research Laboratory, Chilean Nitrate Agricultural Service, Wageningen. The plants were grown in pots containing about 7 kg quartz sand and, to ensure accuracy of fertilization, the ion-exchange resin, dusarite, was added at the rate of 280 g per pot to supply K, Na, Mg, and Ca ions in the following proportions:

Treatment	K	Mg	Ca	Na
	%	%	%	%
1	6	6	88	0
2	6	6	85	3
3	6	6	82	6
4	6	6	76	12
5	6	6	70	18
6	6	6	82	6
7	6	6	82	6

The basal dressing applied per pot consisted of the following salts:

$CaHPO_4.2H_2O$	1.4 g	Borax	15 mg
$FeCl_3$	50 mg	$(NH_4)_6Mo_7O_{24}$	5 mg
$MnSO_4.4H_2O$	114 mg	$ZnSO_4.7H_2O$	50 mg
$CuSO_4.5H_2O$	100 mg		

In addition, the pots of treatments 1, 2, 3, 4, 5, and 7 received a dressing of 750 mg N in the form of NH_4NO_3, 300 mg of which was applied with the basal dressing and the remaining 450 mg in three dressings of 150 mg each in solution. The pots of treatment 6 received 300 mg of N in the form of $Ca(NO_3)_2$ with the basal dressing and the remaining dressings were the same as those of the other treatments. The pots of treatment 7 received in addition 18 mg K in the form of K_2SO_4 in two split dressings.

All treatments consisted of three pots, each containing three plants. There was therefore a total of nine plants per treatment.

The development of the plants was regular. Better growth was observed at first in the treatments receiving 3 and 6 per cent Na. Subsequently, however, the other plants reached almost the same level of development and the differences were scarcely discernible.

When the plants reached physiological maturity, *i.e.* the normal time of harvesting for retting and fibre processing, they were cut and samples about 2 cm long were taken from the stems of each at 30 and at 50 cm above the collar. The samples were dehydrated, bleached, dipped in Carpeschenko preservative liquid, and paraffin-waxed. Sections 20μ in thickness were then prepared with the microtome, taking them at the same position in the sample in all cases.

RESULTS

A microscopic examination of the sections shows how great the similarity is between the sections taken at 30 cm and those taken at 50 cm above the collar. The accompanying photographs (Plate 1) show the histological characteristics of the fibrous zone in typical sections of plants from Treatments 1 to 5.

Treatment 1 (no Na)

The typical section of plants grown without sodium shows a discontinuous fibrous zone of rather reduced width, with the elements irregularly arranged. There are large areas of parenchymatous tissue between the groups of fibres; the elements of individual bundles are separate, with wide intervening spaces. The bundle is reticulate in appearance. At greater magnification the individual fibres are seen to be somewhat variable both in shape and in size. There

are small, generally polygonal fibres and large irregular ones. All have very large lumens and very thin walls. The cambium is moderately developed.

Treatment 2 (3% Na)

Although there are still noticeable areas of intrusive parenchymatous tissue, the fibrous zone of the typical section is fairly well developed, being about double the width of that of the typical section of Treatment 1. In individual groups of fibres the spaces have completely disappeared and the bundle is compact in appearance. The fibres are fairly regular both in shape and size. Their diameter is in general very large, the lumen small and the walls thick. The cambium is well developed.

Treatment 3 (6% Na)

In the typical section the fibrous zone is completely continuous; the areas of parenchymatous tissue which separated the fibres into groups have entirely disappeared. The width of the fibrous zone is very considerable. Except at occassional points of lesser development it is about double that of the typical section of Treatment 2. Individual fibres are regular and homogeneous, of considerable size and closely pressed together, with a very small lumen and thick strong walls. The cambium is perfectly developed and contains numerous elements in the process of differentiation.

Treatment 4 (12% Na)

The fibrous zone is almost continuous and is of considerable width although distinctly narrower than that of the preceding treatment. The fibres are fairly regular, uniform and moderately developed. Their lumens are however, rather larger than those of the average sections of Treatment 3 and their walls are consequently thinner. The cambium is well developed and is active, with new elements differentiating.

Treatment 5 (18% Na)

The typical section has a discontinuous fibrous zone, either through narrowness or through the presence of parenchymatous areas which penetrate and separate the fibres into groups. The fibres are in rather lax bundles. They are very irregular in shape and size, their lumens are very large and their walls consequently thin.

Treatments 6 and 7 (6% Na)

(In Treatment 6 300 mg of the N was in the form of $Ca(NO_3)_2$; in Treatment 7 18 mg K was applied as K_2SO_4).

Apart from small and unimportant differences the results are similar to those obtained with Treatment 3.

DISCUSSION

The results appear to confirm those obtained in the experiments on flax.

In plants grown in the presence of a moderate quantity of sodium (6 to 12%) the development of the fibrous zone is greater while the fibres are more compact and have thicker walls. Above these rates the favourable effect of sodium becomes less or disappears.

The manner in which sodium acts upon the formation of the fibro-vascular tissues cannot be discussed here but it may be supposed that the element has an indirect effect which, as demonstrated by Lehr and van Wesemael [1], confers greater mobility and therefore greater availability upon phosphorus, an element known to participate in the formation of the fibrous zone.

From the practical point of view it has been shown that hemp plants fertilized with sodium-containing compounds, such as nitrate of soda, are richer in fibres and that the fibres are more resistant and more compact.

SUMMARY

The development of the fibrous zone in hemp plants is favourably affected by a moderate Na-supply (6 to 12% of sum of K, Na, Ca, and Mg ions in the medium).

Sodium is supposed to have an indirect effect on the formation of fibro-vascular tissue by conferring greater mobility and therefore greater availability upon phosphorus.

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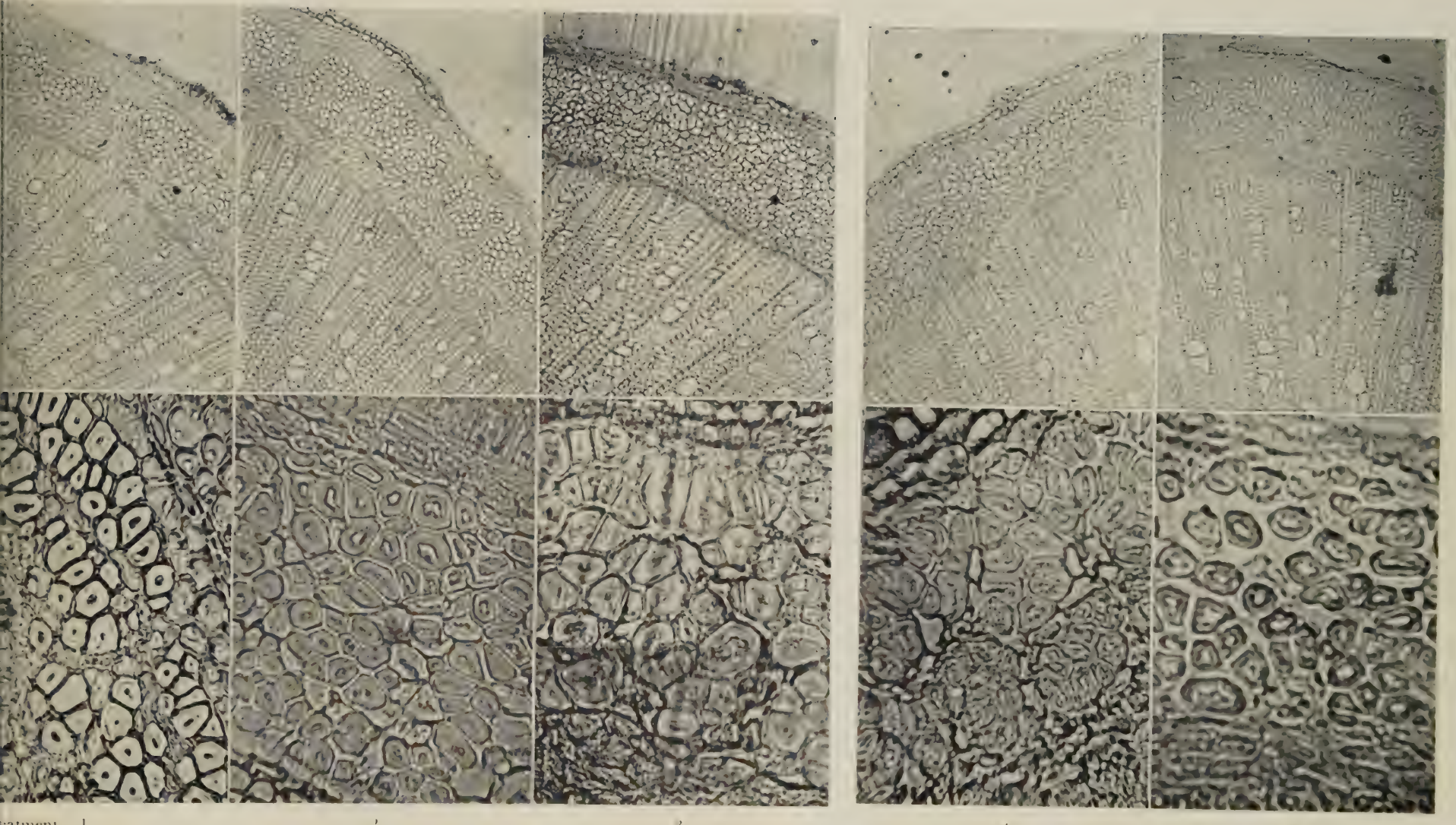

reatment 1 2 3 4 5

te 1. Influence of sodium on the fibro-vascular bundles of hemp.
Treatment 1 — control; 2 — 3% Na; 3 — 6% Na; 4 — 12% Na; 5 — 18% Na.

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BREAKDOWN OF FOREST LITTER IN RELATION TO ENVIRONMENTAL FACTORS

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INTRODUCTION

The rate of litter decay in forests has a pronounced effect on the availability of nutrients for tree growth. In extreme cases litter may be broken down and incorporated in the mineral soil within a year (mull) or it may accumulate for many years on top of the mineral substrate (mor) thus tying up nutrients. Various factors such as geology, topography, climate, soil microflora and fauna, vegetation and leaf properties (pH, buffering capacity, mineral composition, tanning substances, N content or C/N) have been thought to control the formation of mull and mor. These factors have been reviewed and evaluated by Handley [5].

In the present work, which was part of a research program on the rate and mechanism of cycling of matter in forest systems, litter production and breakdown in mull and mor in different seasons and years were compared. The results were related to differences in soil moisture, temperature, microbial population, litter feeding fauna, rates of breakdown of the main leaf components (cellulose and lignified tissue), and the soil concentration of CO_2, the main product of litter decomposition.

MATERIALS AND METHODS

The forest studied has been described previously in detail [7 10]. It was a stand of slash oak, last cut about 1940, locally mixed with birch, alder, and

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poplar, 6.5 to 10.5 m high, 100% cover, timber 1 to 3 $m^3/100m^2$, 27 km ENE of Arnhem, Netherlands.

The main forest-floor types were calcareous mull, pH 7.2, at the lower parts of the area and mor, pH 3.3, at the higher parts. Both are on deep sand to loamy sand with lime in the lowest area.

The understory on the mor area was a Querceto-Betuletum Tx. 1930, 5 to 15% cover, under a canopy of 92% oak (*Quercus robur* L.) and 8% birch (*Betula verrucosa* Ehrh.). On the calcareous mull area there was a Pruneto-Fraxinetum Oberdorfer 1953, 70 to 100% cover, under 28% oak, 36% birch, 19% alder (*Alnus glutinosa* (L.) Gaertn.) and 17% poplar (*Populus tremula* L.)

Litter production from trees and shrubs was measured by periodic litter collections from six bags of cheese cloth with a round opening of 0.5 m^2 each, which were suspended above the forest floor. The collected litter was air dried, sorted, and weighed.

The weight of herbs and grasses annually returned to the soil was estimated by mapping the August vegetation of the whole area in six density classes. In each class three areas of 1 m^2 each were harvested and the air-dry weight of the vegetation was determined. *Anemone nemorosa* L., which disappeared before August, was measured in the same way in May.

Disapparance of fresh litter from the forest floor was determined in each type by comparing the dry weights of fresh litter from six random circular 0.5 m^2 areas in December with the remainder of this litter before the next leaf fall in September. More detailed observation on the disappearance of litter was made by bimonthly weighing, in the field, of the fresh litter from three previously cleaned 1 m^2 quadrats in each type. About 10 per cent of each sample was taken to the laboratory, analyzed for botanical composition and moisture content, and returned to the field.

Decomposition of cellulose was measured by the weight loss of three pieces of filter paper (4 × 4 cm, about 0.4 g each) kept between litter and mineral soil for 6 to 8 weeks. Each piece was wrapped in one layer of 90-gauge nylon mesh to exclude most faunal elements.

Decomposition of lignified tissue was measured by the loss of weight of six sticks (10 × 0.5 × 0.5 cm, about 0.6 g each) of lime wood (*Tilia* sp.) kept vertically in the mineral soil for 6 to 8 weeks. The cellulose content of oak leaves was determined by the difference in carbohydrates, before and after treatment of the material with 75% H_2SO_4, by the anthrone method [8]. The lignin content of oak leaves was determined by the glycocollic acid method [6].

Carbon dioxide was determined by drawing soil air from permanent glass frameworks (65 × 15 × 4 cm inserted 2 cm into the soil) with a battery-operated pump at a rate of 22 l/h. During suction an airtight glass lid covered the frame. After an initial pumping period of 30 minutes which allowed for equilibration, the air was passed for two runs of 1 h each through three washbottles, each containing 8 ml of 0.2 *N* $Ba(OH)_2$. The neutralized $Ba(OH)_2$ was determined titrimetrically with 0.895 *N* oxalic acid.

The following determinations of the microflora of the soil were made: all mushrooms appearing in the area were collected and identified, mycelium concentration was measured by the soil-suspension slide method [10], mycelium growth was determined on five cholodny slides [1] left for 7 days between litter and mineral soil, fungal plate counts were made by enumerating fungal propagules developing from soil suspensions on peptone-dextrose agar containing 33 ppm of bengal rose and 30 ppm of streptomycin to suppress bacterial growth, bacteria and actinomycetes were counted on nutrient-agar plates. Soil for plate counts and soil-suspension slides was sampled in both forest-floor types by taking ten soil cores, 2 cm^3 each, from the surface of the mineral soil.

The litter and soil fauna were determined by bimonthly extractions of eighteen litter and eighteen soil samples, 1 dm^2 each, on berlese funnels. The soil samples were taken from the top 5 cm. The earthworm population was determined by monthly collections from a $\frac{1}{2}$ m^2 area, of all worms which emerged from their burrows while the soil was vibrated for at least 5 minutes with a manure fork. In each forest-floor type each time seven areas were sampled.

Air temperatures and precipitation in the open field were obtained from a station of the Royal Dutch Institute for Meteorology (KNMI), 8 km north of the experimental area. Soil temperatures between litter and mineral soil in the forest were recorded with a thermograph. Precipitation under the tree canopy was measured with a rain gauge. Moisture content of the litter was determined every 2 weeks from eight 1-dm^2 samples from each forest-floor type. Moisture content of the mineral soil was determined weekly in both types from five 10-ml cores from the top 2 cm.

RESULTS

Litter production

The total amount of litter falling from trees and shrubs did not differ greatly in mull and mor (Fig. 1). The mean annual litter production in the two types, based on the measurements of the leaf fall in 1956 up to and including 1959 was 329 g and 356 g respectively. The greater part of the litter consisted of leaves (73 to 79 per cent, depending on type and year). The other part, twigs, seeds, fruits, insect droppings, *etc.*, deserve attention as part of the general budget of organic matter but was excluded from this study. The total amount of leaves shed in the mull type was much smaller in the extremely dry and warm year 1959 than in 1958. This was due to the fact that alder, birch, and poplar produced in 1959 only 58, 61, and 79 per cent resp. of the amount in 1958. The difference in the amount of fresh litter was less pro-

nounced in the mor, due to the dominance of oak, which produced only some percents less. The leaf fall for each of the four main species in the mull type had a characteristic pattern (Fig. 2). Alder showed two peaks, one in July (green leaves) and one in October (brown leaves). Birch fell in August, September, and

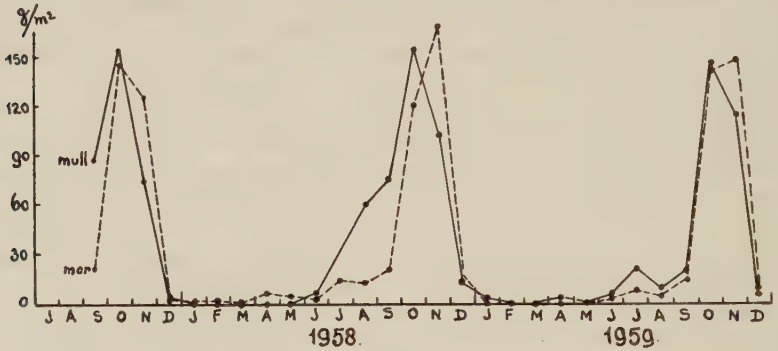

Fig. 1. Total tree litter production in mull and mor.

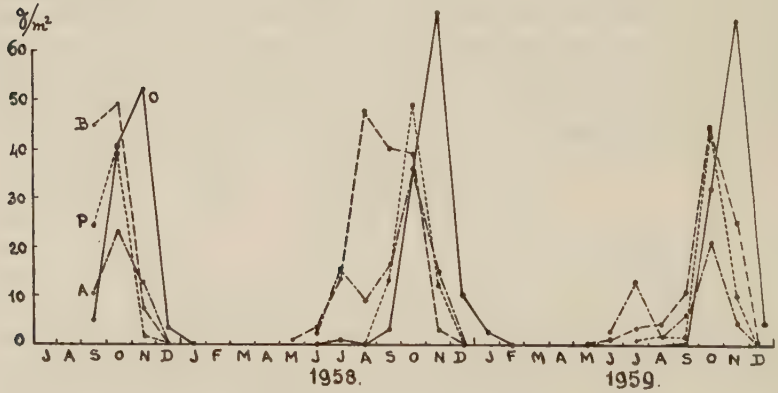

Fig. 2. Litter production of the main species in mull. 0 = oak, B = birch, P = poplar, A = alder.

October, poplar in October, and oak in November. The dry summer of 1959 caused a delay in the leaf fall especially of birch and poplar leaves. In a mixed stand, as in the mull type, this sequence of litter fall determines the structural composition of the litter layer. Dry weight of the herbs and grasses added annually to the forest floor varied from 10 to 200 g/m². The mean values for the mor and the calcareous mull were 14 and 83 g/m², respectively.

Breakdown

The rate of disappearance of litter was high in 1956 and 1957. In both types less of the previous year's litter remained in September 1957 than in December 1956 (Fig. 3). The total amounts of litter which disappeared in both types were about 400 g/m². From

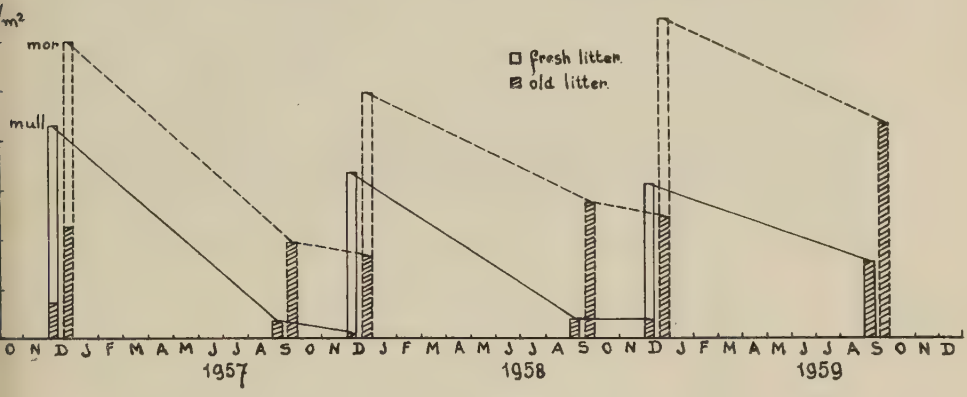

Fig. 3. Rate of disappearance of litter in mull and mor.

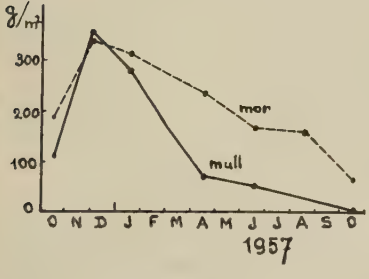

Fig. 4. Amount of litter on a cleaned surface in mull and mor.

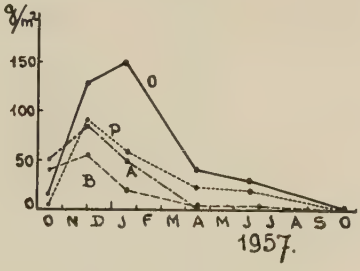

Fig. 5. Amount of fresh oak (O), birch (B), poplar (P), and alder litter (A) in mull.

December 1957 until September 1958 these amounts were 300 and 200 g/m² for mull and mor respectively, and consequently, especially in the mor, the weight of remnants of old litter was much higher than that in December of the preceding year. In the dry year 1959, the decomposition was even slower than in 1958 *viz* 150 g/m² in the mull and 200 g/m² in the mor. This resulted in a net accumulation of litter in both mull and mor. The disappearance of the litter which fell in autumn 1956 is represented in Figure 4. During

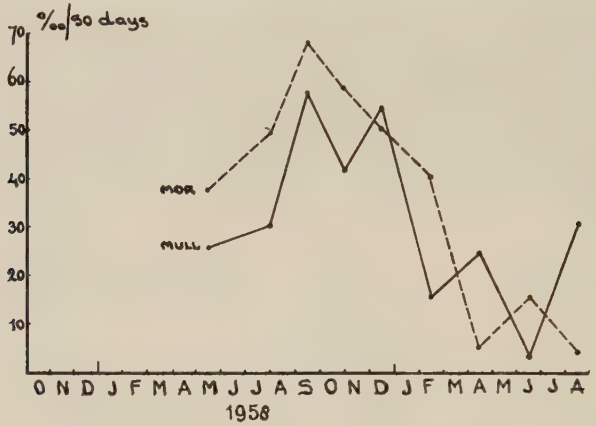

Fig. 6. Loss of weight of filter paper in mull (———) and mor (– – –).

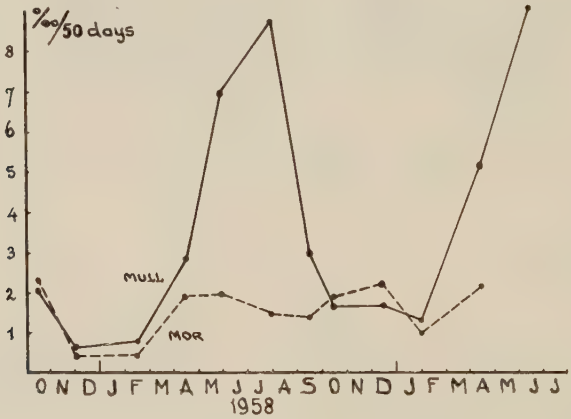

Fig. 7. Loss of weight of wooden sticks in mull and mor.

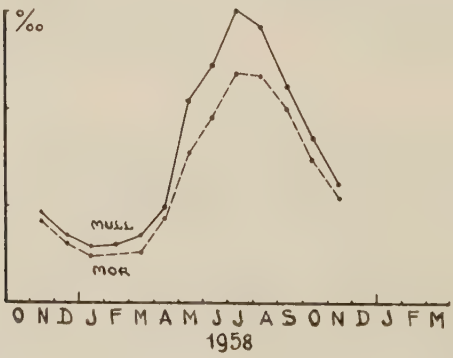

Fig. 8. Concentration of CO_2 in soil air in mull and mor.

the first 4 months the decrease of litter was very rapid in the mull type and slow in the mor. The reverse was the case in the remaining part of the year. The rates of decrease in 1957 of the main litter species in the mull type are given in Figure 5. Birch and alder disappeared more quickly than did oak and poplar.

In both mull and mor the loss of weight of filter paper was much higher than that of lignified tissue (Figs. 6 and 7). Rates of disappearance of the paper reached maxima in the fall and were in general somewhat higher in mor than in mull. The rate of disappearance of wood in mull showed a maximum in summer and a minimum in winter. In mor this rate fluctuated less with lowest values in winter. Figure 9 shows that in fresh oak litter from mull

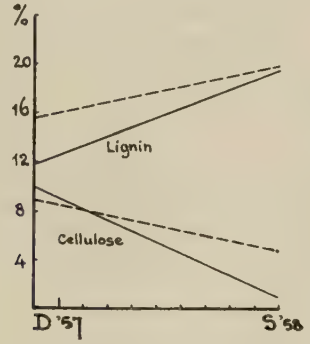

Fig. 9. Lignin and cellulose content in oakleaves fallen in 1957. ———— mull, – – – – mor.

there was more cellulose and less lignin than in oak litter from mor. The decrease of the cellulose content and consequently the relative increase of the lignin content in oak leaves from mull was larger than in oak leaves from mor. The concentration of CO_2 in the soil air was accepted as an indication of over-all microbial activity because carbon dioxide is the main end product of aerobic decomposition. Monthly averages of the measurements of CO_2 concentrations were highest in summer (Fig. 8). There was consistently more CO_2 in mull than in mor.

Biota

From Figures 10 to 13 it appears that all measures of the fungus flora reached higher values in mor than in mull, whereas numbers of bacteria and actinomycetes were higher in mull. Development

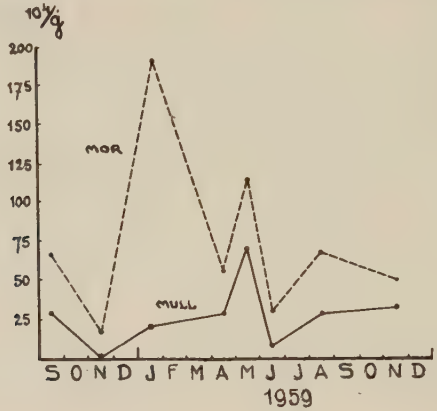

Fig. 10. Plate counts of fungi from mineral soil in mull (———) and mor (– – –).

Fig. 11. Mycelium development on cholodny slides in mull and mor.

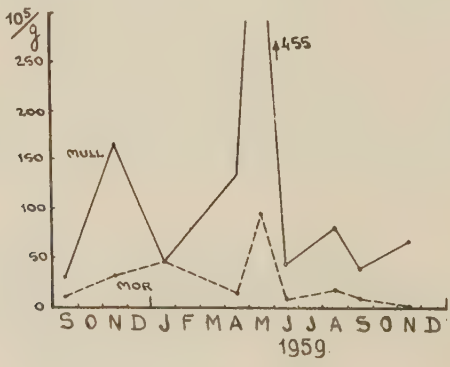

Fig. 12. Plate counts of bacteria and actinomycetes from mineral soil in mull and mor.

of mycelium and bacteria reached a peak in spring and a low level in winter and summer. Mycelium concentration and plate counts of fungi reached maximum values in winter, whereas maximum numbers of mushroom species occurred in the fall. Mycelium concentration showed one cycle annually with maxima in winter and minima in summer.

The density of some of the most important litter feeding insects in different seasons shows a definite decrease in summer (Figs. 14 and 15). The only earthworm species, frequently caught by vibration, was *Lumbricus rubellus* Hoffm., the catches of which are given in Fig. 16. In both forest types captures of this species decreased in spring and increased in autumn. The density was always higher in mull than in mor. In the dry year, 1959, the activity of this surface species was very low.

DISCUSSION

Seasonal fluctuations

The detailed observations on the biota and their activities for at least 13 months make it possible to construct a picture of the mechanism of the litter breakdown from one leaf fall to the next.

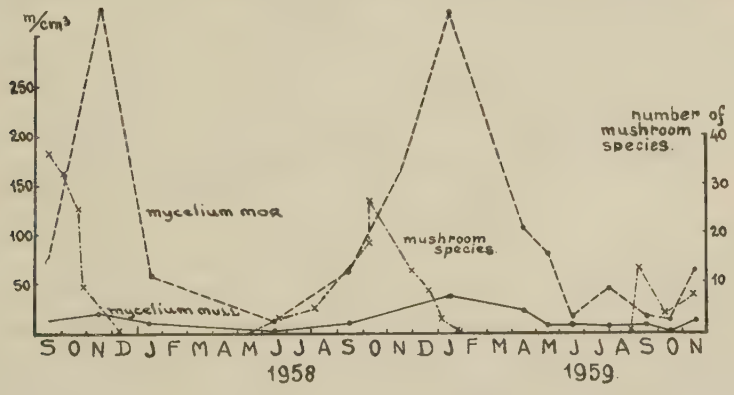

Fig. 13. Mycelium concentration in mineral soil in mull (———) and mor (– – –) and total number of mushroom species.

The maximum litter fall occurred in October–November (Fig. 1) when temperatures were still about 10°C (Fig. 17), mycelial growth on the cholodny slides was rapid (Fig. 11), mushrooms appeared in maximum numbers and the accumulation of mycelium was underway (Fig. 13). Bacteria multiplied rapidly [10]. Their numbers

in the top 2 cm of the mineral soil were high in November (Fig. 12). The loss of weight of wooden sticks in the soil (Fig. 7) and the CO_2 production (Fig. 8) declined rapidly under the influence of decreasing temperatures.

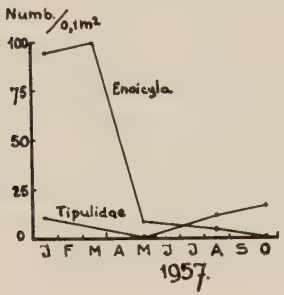

Fig. 14. Density of some saprophagous insect larvae in mull.

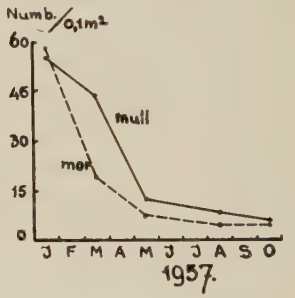

Fig. 15. Density of Nematocera-larvae (excl. Tipulidae) in mull (———) and mor (– – –).

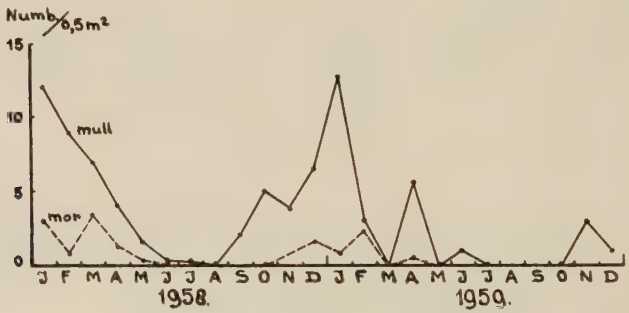

Fig. 16. Numbers of *Lumbricus rubellus*, activated by shaking the soil.

In December and January, mycelium (Fig. 13) and the concentration of fungal propagules (Fig. 10) increased to reach a maximum in January. From then on, all microbial activity was low, presumably because of low temperatures. Nevertheless, during the first 4 months of the year the amount of litter in the mull decreased at a relatively rapid rate (Fig. 4). This reduction may be caused by leaching, by the superficial attack by great numbers of enchytraeids which move on the wet leaves and by larger burrowing saprophagous animals, like nematocera larvae (*Tipulids* and *Bibionids*), the earthworm *Lumbricus rubellus* Hoffm. and several species of slugs and snails. The larvae of the caddis fly *Enoicyla*

pusilla Burm. was very numerous in 1957 and took an active part in the desintigration of the litter [4] (Figs. 14, 15).

With rising temperatures in May bacterial and fungal numbers and mycelium growth increased but animal numbers decreased. The decomposition of cellulose and wood increased. This is reflected in the increasing CO_2 content of the soil atmosphere. Often in late May a dry period occurred and microflora and fauna minimized their activity in and also directly under the litter. In the mineral soil which was still moist, the decomposition of wood and the production of carbon dioxide by micro-organisms and plant roots

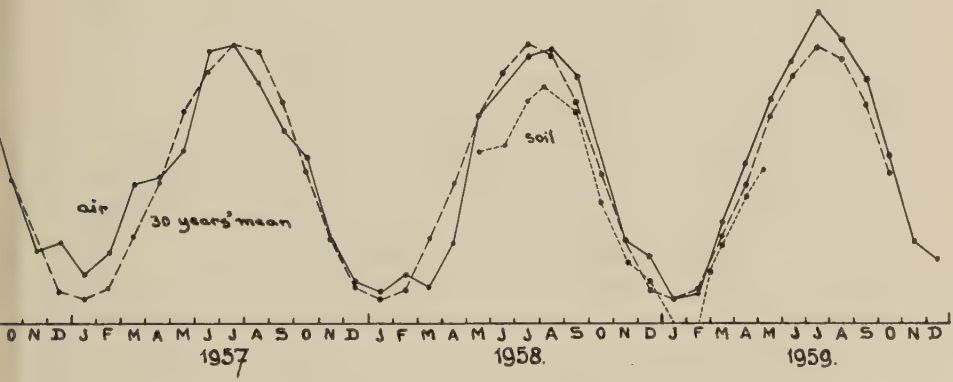

Fig. 17. Monthly averages of air temperature, soil temperature and the 30 year's mean of air temperature.

increased with rising temperatures. Summer rains, usually starting in late July or August, caused maxima in all microbial activities and an increase in animal activities. Thus the new leaf fall met an active population in the soil. In the mull the decomposition of filter paper strips just below the litter layer showed a maximum in September (Fig. 6). This possibly was caused by leachate from early fallen fresh litter which supplied easily decomposable nitrogeneous substances and prevented desiccation.

These observations emphasize the overriding influences of seasonal changes in temperature and moisture on the decomposition process in the forest floor. In general, high temperature and favourable moisture condition stimulate populations, their activities, and the resultant CO_2 production. In the case of the activity of

larger saprophagous animals and mycelium accumulation, other mechanisms prevail. The previous observations also indicate the difference in effect of the seasonal climatic changes in the different layers. In the deeper soil, CO_2 production and decay of wood were strictly controlled by temperature, whereas in the top 2 cm decomposition was governed both by temperature and moisture, as was displayed by microbial populations and growth.

Mull and mor

In general the fluctuations in numbers of organisms, breakdown rates, and CO_2 production were synchronous in mull and mor. This synchronism points to a similar influence of meteorological conditions in both mull and mor. The levels of these fluctuations, however, were different. Numbers, growth, and accumulation of fungi were constantly higher in mor than in mull, whereas the numbers of bacteria and actinomycetes were higher in mull (Figs. 10 to 13). The numbers of the larger litter-feeding animals (snails, earthworms, millipedes, isopoda, and insect larvae) were much higher in calcareous mull than in mor, but microarthropods (mites and springtails) were consistently more numerous in mor [2 3]. Due to the activity of the larger litter-feeding animals the litter in the calcareous mull disappeared quickly. In 1957, at the beginning of the dry period in April, only about 20 per cent of the total amount of litter which fell in 1956 remained (Fig. 4). By then about 70 per cent of the corresponding litter in the mor was still present. The small amount of resistant litter fragments in the mull decreased slowly after that, but in the mor the decrease continued at a constant rate.

The decomposition of cellulose (Fig. 6) was about equal in mull and mor. The loss of weight of wooden sticks (Fig. 7), however, showed quite different curves for the two types; the high peaks in decomposition rate from June until August in mull were absent in mor.

Warburg experiments established that litter extract added to undisturbed soil was broken down twice as fast in mull as in mor. This correlates well with the higher concentration of carbon dioxide, the more rapid loss of weight of wooden sticks, and the faster breakdown of cellulose in oak leaves in mull. According to Went [9] who worked in the same forest, decomposition of cellophane strips

in the mineral soil was consistently faster in calcareous mull than in mor. In contrast we found that loss of weight from filter paper strips on top of the mineral soil was more rapid in mor than in mull. The relatively slow decomposition of the paper strips in the mull may have been caused by its frequent desiccation. The influence of moisture also was demonstrated by the difference of the loss of weight of filter paper in calcareous mull and mor during May, June, and July, 1958, and in the same period in 1959 (Fig. 6). Precipitation during those periods was 250 and 110 mm, respectively (Fig. 18).

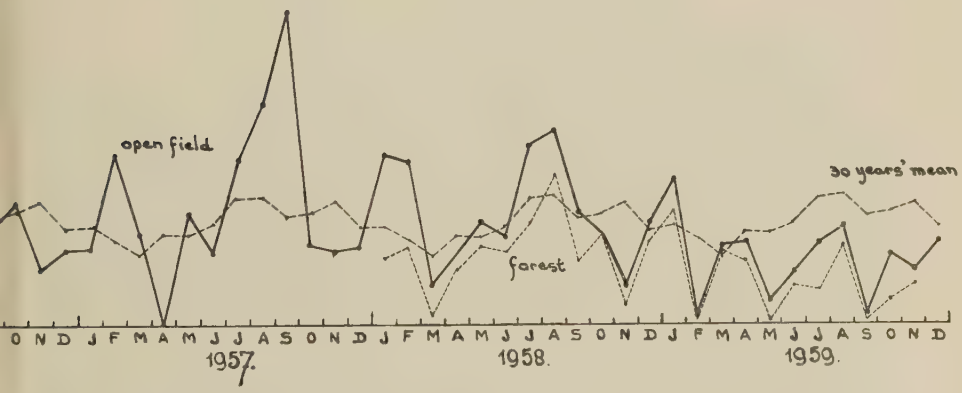

Fig. 18. Monthly rainfall in open field and forest and the 30 year's mean of rainfall in open field.

The mean annual leaf fall in mull and mor was only slightly different (see p. 297). However, there was a qualitative difference, as in the mull the C/N of all fresh litter was 27.8 as compared to 33.2 in mor. This difference was mainly due to the presence of a profuse ground cover and of alder in the mull. In addition to this oak litter in mull consisted mainly of shadow leaves and in mor mainly of sun leaves. Sun and shadow leaves do not only differ in lignin content (19.4 and 15.9 per cent resp.) but also in form and structure which resulted in a more rapid attack of the shadow leaves by soil animals [2]. There was also a difference in the time of litter fall as litter fell earlier in mull than in mor. At last it must

be mentioned that the evaporation above the litter in mor was twice as high. This was caused by a higher temperature and a lower vapour pressure inducing a higher saturation deficit. Especially in spring the litter began in desiccating more rapidly in mor and so the circumstances for animal attack are worse. These differences inducing greater animal activity during autumn, winter and spring with subsequent increased microbial decomposition [4] in the mull caused the litter to disappear initially more rapidly in this type. Consequently, during the summer only a thin, discontinuous litter layer remained in the mull leaving the mineral soil subject to desiccation which interrupted animal and microbial activity. In the mor litter breakdown was more constant throughout the year (Figs. 4 and 7).

Annual differences

Comparison of the microbial population and its activity in different years showed differences in phase as well as in level. In 1958, winter and spring temperatures were lower (Fig. 17) and summer was drier than in 1957 (Fig. 18). Consequently, decay of the litter in both mull and mor was slower in 1958 (Fig. 3). This led to a considerably larger amount of undecayed litter in the mor at the end of the season of 1958. In 1957 most of the litter in the mull had disappeared from the surface by May. In 1958 breakdown by animals in winter and spring was slower than in 1957 and was taken over by micro-organisms until the end of the season. By then only a small amount of litter remained.

Precipitation was extremely low in 1959: 512 mm against 825 mm in 1958 and 907 in 1957. This resulted in litter accumulation, both in mull and mor (Fig. 3).

The difference between 1957 and 1958 in appearance of mushrooms and in the number of species present (Fig. 13) were also due to differences in precipitation. In mid-August 1957, summer rains fell after a rather dry spring. As a result an abundant flora of mushrooms thrived, which lasted until the end of September. In 1958, however, with relatively dry weather in August and September, mushrooms appeared in October in smaller numbers representing fewer species than in the previous year. This shift in phase can also be seen in the curve representing concentration of mycelium (Fig. 13).

The influence of drought was illustrated during the summer of 1959 when the moisture content in mull and mor dropped below the wilting point (Fig. 19). Consequently, the values obtained for mycelium growth, mycelial concentration, plate counts of fungi as well as bacteria and actinomycetes, numbers and species of mushrooms, cellulose decomposition, CO_2 production, animal

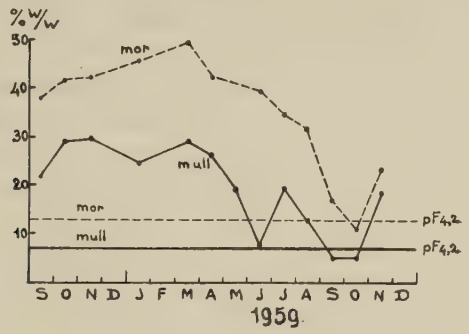

Fig. 19. Monthly averages of moisture content in soil in mull and mor.

activity and disappearance of litter were lower in 1959 than in 1958. After adequate rainfall in November 1959, the microbial population increased again.

The annual differences in litter breakdown in both mull and mor were caused mainly by differences in amount and distribution of rainfall and to a lesser extent by variations in temperature. The mull was more vulnerable to desiccation to the wilting point than mor, especially in the summer when the litter layer in mull was thin and discontinuous. Rapid desiccation and the dominance of surface-rooting species in the mull provided a built-in regulating mechanism against litter accumulation which produced less litter during times of slow decomposition. In the mor type the deep-rooting oak dominated, and drought did not influence litter production. Here a full year of breakdown was required to balance litter fall. Slightly unfavourable wether conditions would cause litter accumulation. In the mull only extremely unfavourable conditions can cause litter accumulation, which will be eliminated during the next more favourable years.

Topographic and climatic factors, the composition of fresh litter, and the biota governed the rate of litter disappearance whereas the

latter two factors also determined the qualitative aspects of decomposition. The microflora and the soil fauna appeared to be determined primarily by the composition of the litter, that is by the botancial composition of the vegetation, by the rate of desiccation of the litter layer, and locally by the presence of lime [2 10]. Minderman [7] indicated a strong correlation between forest-floor type and water regime in the same stand. It then seems plausible to state that in this area differences in depth of the water table, water-holding capacity and the content of lime in the soil caused differences in vegetation and subsequent differences in microflora and litter-feeding soil fauna, and ultimately in forest-floor type. The correlation between forest-floor type and vegetation in this area has been established and will be dealt with in a subsequent paper.

SUMMARY

Production and breakdown of forest litter in a single stand were compared in different forest-floor types (mull and mor), during several years. Litter production was almost equal in both types. In the extremely dry year 1959 less litter was produced in the mull, due to a strong desiccation and surface-rooting species. In the mor, the litter production was less influenced, thanks to lesser desiccation of the soil and the deeper rooting oaks. The litter in the mull fell earlier and had a more favorable C/N for biological decomposition (27.8) than in mor (33.2).

The fluctuations of density and activity of the floral and faunal elements taking part in the breakdown of litter in mull were generally synchronous with those in mor. Between these two types, however, differences in level of density and activity of the biota existed. In general, biological activities and carbon dioxide concentrations in the soil were higher in calcareous mull than in mor. In the calcareous mull larger litter-feeding animals, bacteria, and actinomycetes dominated as a result of the higher air humidity, the presence of calcium carbonate and the larger amounts of protein and easily decomposable carbohydrates in the mull litter. In the mor, where the litter contained more lignin and cellulose and less protein, fungi and microarthropods were most numerous. The larger saprophagous animals were mostly active from October to April. This caused a rapid disappearance of the freshly fallen litter in the calcareous mull, followed by incorporation of the breakdown products into the soil. This breakdown was mainly mechanical and thus did not yield high carbon dioxide concentrations in the soil. As temperatures rose, the microbiological populations and their activities increased, depending on moisture conditions. In the calcareous mull the annual breakdown of shed litter was almost completed in half a year. In

mor this breakdown continued through a full year. Unfavourable weather conditions, as in 1958, retarded decomposition, prolonged the period required for breakdown in the calcareous mull, and caused accumulation of litter in the mor. Extreme drought in 1959 caused a net accumulation even in the calcareous mull. In an acid mull type the values obtained for practically all factors and processes studied in the calcareous mull and the mor occupied an intermediate position. It is concluded that differences in forest-floor type in the area of the present work were primarily conditioned by edaphic factors which influence vegetation, litter composition, air humidity, soil microflora and fauna, and ultimately forest-floor type.

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TRANSLOCATION OF POTASSIUM AND SODIUM IN INTACT MAIZE SEEDLINGS

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INTRODUCTION

In a previous publication (Bange [2]) it was shown that two mechanisms are involved in the absorption of K by intact maize seedlings. One of these mechanisms is hardly selective, transporting K as well as Na with little, if any, preference. On the other hand the second mechanism is highly selective and, though it may transport some Na in the absence of K, it does not do so if any K is present in the root environment.

The intriguing question is: what can be the physiological background of this twofold mechanism of K absorption? Is there a more or less permanent separation inside the tissue between K-ions taken up by either mechanism or is all K absorbed delivered to one common pool in the root?

Sutcliffe [6] found that in intact barley plants the preference for K over Na is much more pronounced than in excised barley roots. At the same time K was transferred to the shoot to a much larger extent than Na. As a tentative explanation he suggested that the transfer of K to the shoot is controlled by a more selective mechanism than the accumulation in the vacuoles of the root cells.

Though K- and Na-absorption in barley obeys other laws than in maize (Epstein and Hagen [4]), it seemed profitable in view of these results to examine whether in maize the specific mechanism of K-absorption is especially concerned with the transfer of K to the shoot and the non-specific mechanism with the accumulation of K and Na in the vacuoles of the root cells. This was the purpose of the experiments described below.

MATERIAL AND METHODS

a. *Plant material*

The experiments were performed with the same hybrid (single cross 9 × D) as used in the previous work. The seed (harvest 1958) was obtained from the College of Agriculture, University of Wisconsin, Madison, U.S.A.

Sets of 10 seedlings were raised in an air-conditioned room (temperature 25°C) according to a rigourous scheme as follows.

About 150 seeds were disinfected with 1% $HgCl_2$ solution for 2 minutes. After soaking in aerated, demineralized water for 24 hours the seeds were allowed to germinate in the dark on a layer of wet cotton-wool between two sheets of filter paper. After 66 hours sets of 10 seedlings were mounted on coarse nylon netting spread in the centre of a ring of flexible plastic tubing. These "units" were then placed on 400-ml glass beakers filled with about 230 ml of culture solution so that the level of the solution was just below the netting. In this way a set of 9 units of 10 seedlings each was prepared from each batch of 150 seeds.

The set was then placed in an artificial-light chamber containing 12 fluorescent lamps (Philips types 40 W/55 and 40 W/33 alternately) per square meter. The distance between the lamps and the plants amounted to about 50 cm. The daily light period was 16 hours.

For optimal development it appeared to be useful to place the seedlings in a very humid atmosphere during the first 4 days of their stay in the light chamber. Therefore during this time a piece of wet aseptic gauze was put over the set and covered with a sheet of transparent plastic.

The culture solution consisted of 0.25 mM $Ca(H_2PO_4)_2$ + 0.25 mM $Mg(NO_3)_2$. On the tenth day of their stay in the light chamber a dilute complete nutrient solution prepared according to Woodford and Gregory [7] and with micronutrients added was supplied to the seedlings. After 24 hours this solution was replaced by 10^{-4} M $CaSO_4$, the seedlings remaining in this solution until they were 20 days old and used in the experiments. Both the culture solution and the $CaSO_4$ solution were renewed every 24 hours except on Sundays. Where two sets of plants a week were raised it was necessary to introduce artificial Sundays in order to get strictly comparable material. All beakers were aerated continuously.

The fresh weight of the leaves of one unit of 10 seedlings amounted to about 7.5 grams and of the roots to about 10 grams.

Though the method of raising the seedlings deviated in some respects from the procedure followed previously, no differences either in appearance or in physiological behaviour were observed as compared with the earlier material (*cf* Bange [2]).

b. *Experimental planning*

The time course of K and/or Na absorption and distribution between root and shoot was followed at 3-hour intervals for 24 hours in 5 series of experiments. In the first series Na and in the second series K was the

only cation supplied in the solution, the concentration in both cases being 2 me/l. (milliequivalents per litre). In Series III both K and Na were supplied, K in a concentration of 0.1 me/l. and Na in a concentration of 2 me/l. Series IV and V were identical with Series II and III respectively with the exception that in these series one half of the plants was transferred to demineralized water and the other half to 3×10^{-4} *M* $CaSO_4$ solution after 12 hours.

In spite of the care taken to raise comparable plant material, it appeared necessary to run each series in quadruplate to reduce the experimental variability to a low level. So altogether $5 \times 4 = 20$ series of experiments were run requiring 20 sets of 9 plant units each.

Where the experiments extended over a period of about 3 months the possibility of a slow shift in the behaviour of the plant material had to be taken into account. Therefore the whole experimental setup was planned in advance in the following way.

From each 9-unit set 4 units were allowed to absorb for periods of 3, 9, 15, and 21 hours in one of the 5 series of experiments. Another 4 units absorbed for 6, 12, 18, and 24 hours in another series. The remaining unit was used to determine the initial K- and Na-content of roots and shoots. In this way each of the 5 experimental series was made up of plant material from 8 different sets, care being taken to insure that these sets were evenly distributed over the 20 successive sets of units raised.

The time of the day at which the 8 units were transferred to the experimental vessels was not the same for all units but was arranged such that the presence of the experimentator was not required from midnight to 8.00 a.m..

c. *Experimental procedure*

Before the start of an experiment the roots were given a rinse in 3 changes of 230 ml of demineralized water (10 minutes/rinse). They were then transferred to the experimental vessels, consisting of 2 glass aquaria covered with sheets of hardboard. The covers had holes to accomodate the plant units.

The aquaria contained 15 litres of experimental solution. All K- and Na-ions were supplied as a mixture of primary and secondary phosphates adjusted to a pH-value of 6. Depletion of these solutions during the experiments was negligible, except in the case of Series III and V where the K-concentration was only 0.1 me/l. Therefore in these series an additional 50 litres of solution was stored in a big glass bottle and circulated through the aquarium at a steady rate.

The temperature of the aquaria was maintained at 20°C by a waterbath. Light conditions during the experiment were identical with those described for the cultivation period with the obvious exception that no dark period was inserted. A fan maintained a moderate air circulation around the shoots. Transpiration did not exceed 1 ml/hour per unit.

Immediately after the experiment the roots were rinsed in 2 changes of 230 ml of cooled $CaSO_4$ solution (10^{-3} *M*) for 5 minutes each in order to remove K- and Na-ions present in the free space. However, in Series IV

and V this final rinse was omitted for plant units that had been transferred to demineralized water or 3×10^{-4} *M* $CaSO_4$ solution. Instead these units were given a rinse in 2 changes of 230 ml of either of these media (5 minutes each) prior to the transfer to the small 7-litre aquarium used for this experimental treatment.

Subsequently root and shoot were separated by cutting the mesocotyl and dried under infra-red lamps.

d. *Analytical methods*

Charring of the dried material was accomplished by the addition of approximately 3 ml of a mixture of 475 ml ethanol (96%) and 25 ml H_2SO_4 (98%) followed by ignition. This procedure appeared to be advantageous for obtaining a white ash in the subsequent dry ashing. The latter was carried out at 550 to 600°C in a muffle furnace for about 14 hours.

The ash was dissolved by adding a few mls of dilute HCl to the porcelain crucibles and digested on a hot plate for about 20 minutes prior to filtration. The filtrate was made up to 100 ml and subsequently diluted 10 times for flamephotometrical analysis.

A Model DU Beckman flame spectrophotometer with photomultiplier attachment was used. Using a split width of 0.03 mm, interferences between K- and Na-emission were negligible at the prevailing concentrations (between 0 and 0.5 me/l).

EXPERIMENTAL RESULTS

Series I: *Absorption from a solution containing 2 me/l Na* (see Fig. 1).

Attention should be given to two important features. In the first place the overall rate of Na-absorption shows a gradual decline with time, especially during the first 12 hours. In the second place hardly any Na is transported to the shoot within a period of about 15 hours. After that the Na-content of the shoot slowly rises.

Series II: *Absorption from a solution containing 2 me/l K* (see Fig. 2).

In sharp contrast to Na absorption the overall rate of K uptake appears to be strictly linear with time. As in the case of Na the rate of accumulation in the root gradually decreases, especially during the first 12 hours, but at the same time there is an appreciable and increasing rate of transfer of K to the shoot. It should be noted that the K-content of the shoot does not start rising before 6 hours.

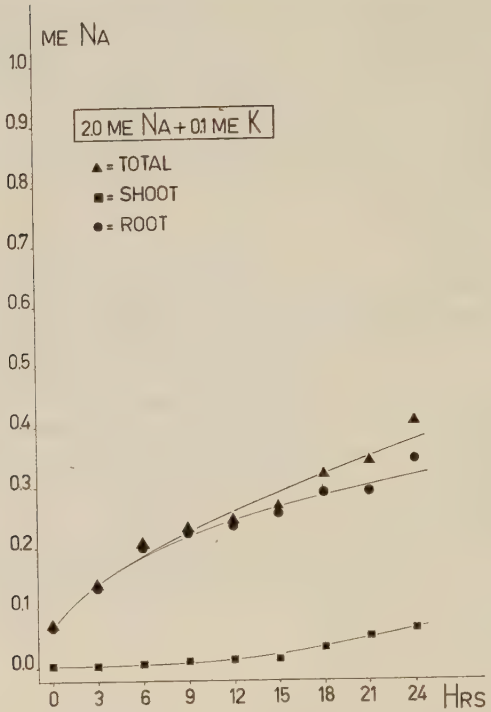

Fig. 1. Total Na-absorption from a 2.0 me/l Na-solution and distribution over root and shoot in relation to time.

Series III: *Absorption from a solution containing 2 me/l Na + 0.1 me/l K* (Figs. 3 and 4).

The overall rate of K-absorption (Fig. 3) shows a slow initial rise and does not become steady before 9 hours. During the same period the rate of K-accumulation in the root is constant, but after 9 hours it starts declining to become next to zero after about 18 hours. After 3 hours the K-content of the shoot starts rising, the rate of transfer not becoming steady before about 18 hours. Then essentially all K absorbed is transported to the shoot.

Na-absorption (Fig. 4) shows only minor deviations from the behaviour in the absence of K. In the first place the rate of absorption during the first 9 hours is somewhat lower. In the second place the time for the appearance of Na in the shoot is shortened 3 to 6 hours from the previous case.

Series IV: *Absorption from a solution containing 2 me/l K for 12 hours and subsequent transfer to a K-free medium for the same length of time* (Fig. 5).

There is no net loss of K after the transfer to the K-free medium, the line for overall absorption becoming parallel to the abscissa after 12 hours. At the same time there appears to be a considerable

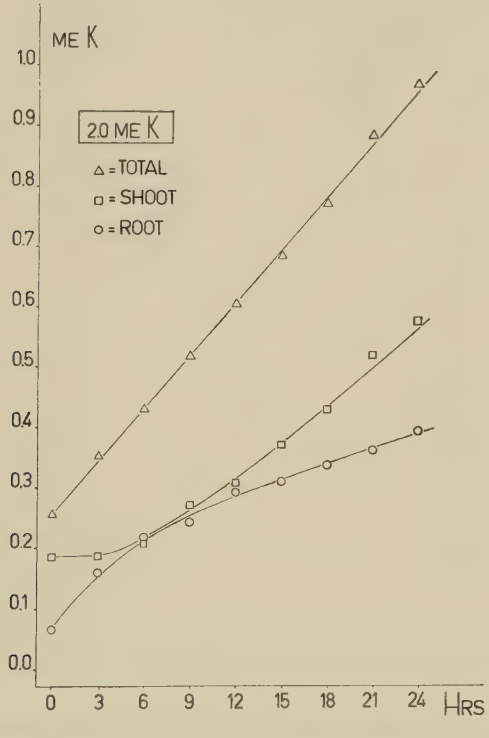

Fig. 2. Total K-absorption from a 2.0 me/l K-solution and distribution over root and shoot in relation to time.

redistribution of internal K during this period, the K-content of the shoot rising at a constant rate at the expense of the K-content of the root. However, it should be noted that the rate of transfer of K to the shoot changes abruptly on transfer to the K-free medium.

No differences in behaviour either in this or in the following series were observed between plant units transferred to demineralized water or to 3×10^{-4} *M* $CaSO_4$ solution.

Series V: *Absorption from a solution containing 2 me/l Na + 0.1 me/l K for 12 hours and subsequent transfer to a Na- and K-free medium for the same length of time* (Figs. 6 and 7).

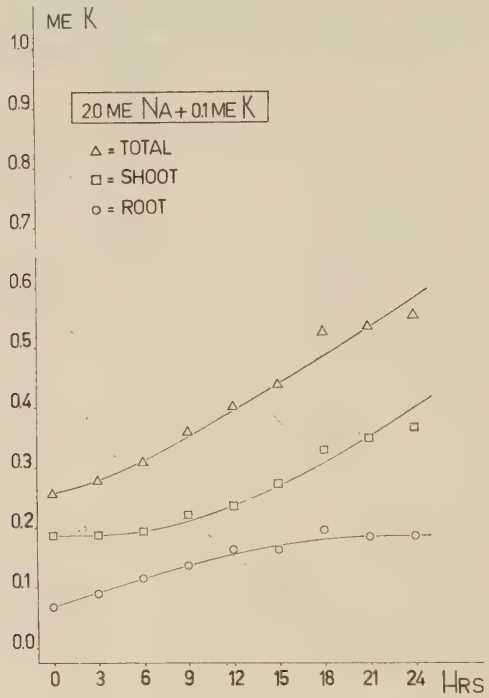

Fig. 3. Total K-absorption from a solution containing 2.0 me/l Na + 0.1 me/l K and distribution over root and shoot in relation to time.

Again a redistribution between root and shoot of K taken up in the first 12 hours takes place (Fig. 6), K being transferred to the shoot at a decreasing rate. No abrupt change in the rate of transfer after 12 hours is observed in this case.

Neither net loss nor redistribution of previously absorbed Na occurs after 12 hours (Fig. 7).

DISCUSSION

The transfer of Na to the shoot within 24 hours appears to be negligible in our maize seedlings as compared to the transport of

K to the shoot during the same time period. This is in close agreement with the results mentioned above of Sutcliffe [6] with intact barley plants. The phenomenon is so much the more remarkable because, as pointed out in the introduction, in maize K absorption is mediated partly by the same mechanism as Na uptake. The obvious conclusion is that a highly specific mechanism must be

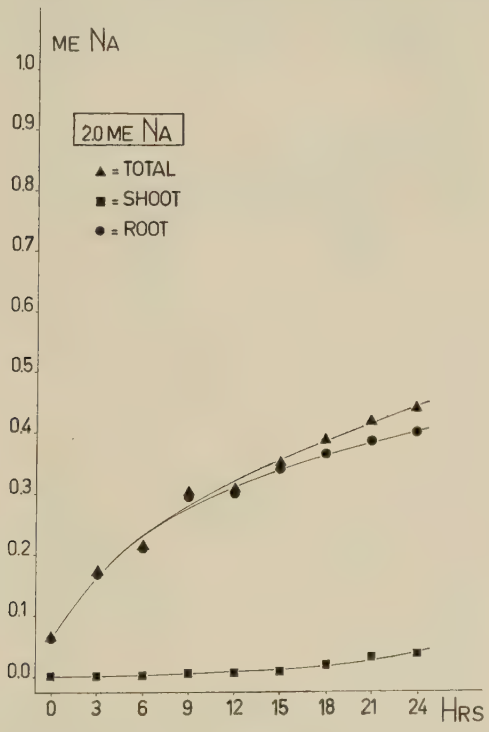

Fig. 4. Total Na-absorption from a solution containing 2.0 me/l Na + 0.1 me/l K and distribution over root and shoot in relation to time.

involved in the transfer of K to the shoot, the effect being much too large in our view to be due solely to purely physicochemical differences between both ions. This high specificity emphasizes the insignificance of any passive component in the transfer of ions to the shoot in our experiments, as does a simple consideration of the average rate of transpiration and the concentrations used.

A specific transfer as mentioned above might take place from a

pool in the roots to which primarily all K and Na absorbed is delivered (*cf* Fig. 8).

There are two reasons why this scheme must be rejected. In the first place it is not clear why the rate of transport of K to the aerial parts should exhibit an abrupt change after transfer of the plants to a K- and Na-free medium (Fig. 5) if a large pool of trans-

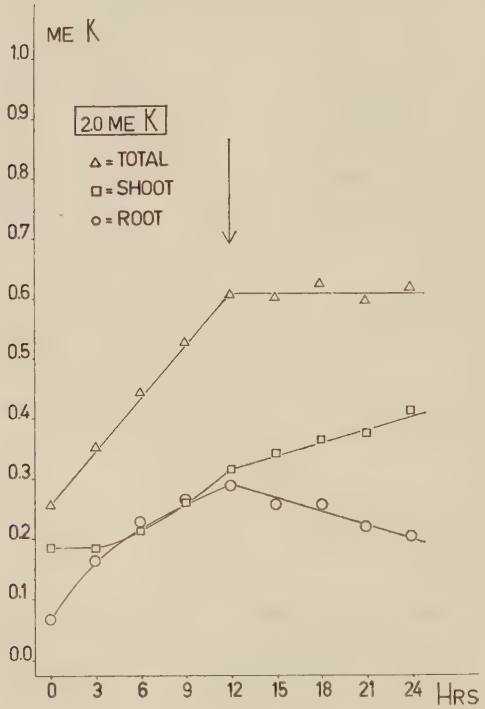

Fig. 5. Redistribution over root and shoot of K absorbed from a 2.0-me/l K-solution after transfer of the plants to a K- and Na-free medium in relation to time.

ferable K is available in the roots. In the second place the scheme does not explain the remarkable linearity of overall K-absorption with time (Fig. 2). If the decline in the rate of Na-absorption with time (Fig. 1) should be attributed to the building up of an internal Na-concentration in the root pool, as it likely should, it cannot be seen why K-absorption should not show the same phenomenon,

the K- and Na-content of the root running almost parallel in the respective experiments (*cf* Figs. 1 and 2).

The only alternative explanation seems to be that there is no common pool but a more or less permanent separation inside the root between K absorbed by the specific mechanism on the one hand and the non-specific mechanism on the other. The question then arises whether K absorbed by either of these mechanisms is equally liable to specific transfer to the shoot.

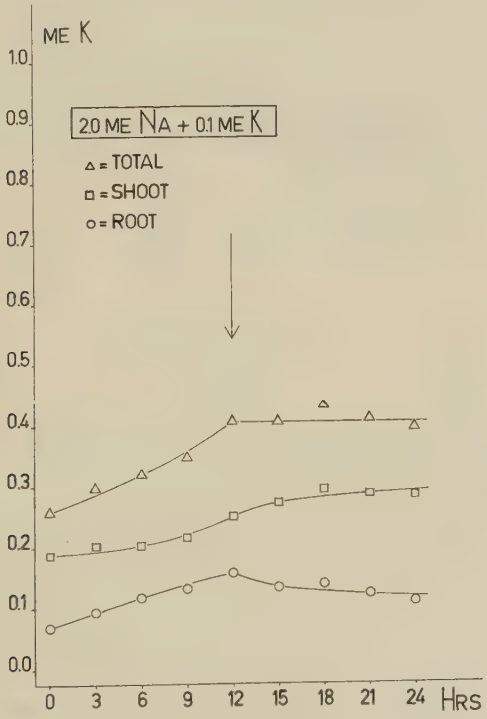

Fig. 6. Redistribution over root and shoot of K absorbed from a solution containing 2.0 me/l Na + 0.1 me/l K after transfer of the plants to a K- and Na-free medium in relation to time.

The experiments of Series III (Fig. 3) in which non-specific K-absorption was eliminated by the addition of excess Na show that after the building up of a relatively low K-concentration in the root within the first 18 hours, essentially all K absorbed is transferred to the aerial parts. So the specific mechanism of K-uptake

seems to be concerned directly with the supply of K for transport to the shoot.

On the other hand the rate transfer of K to the aerial parts in case both mechanisms of K-absorption are operative (Fig. 2) appears to exceed considerably the rate of transfer of K absorbed by the specific mechanism only (Fig. 3). The inescapable conclusion is that K transported into the root by the non-specific mechanism is also transferred to the shoot.

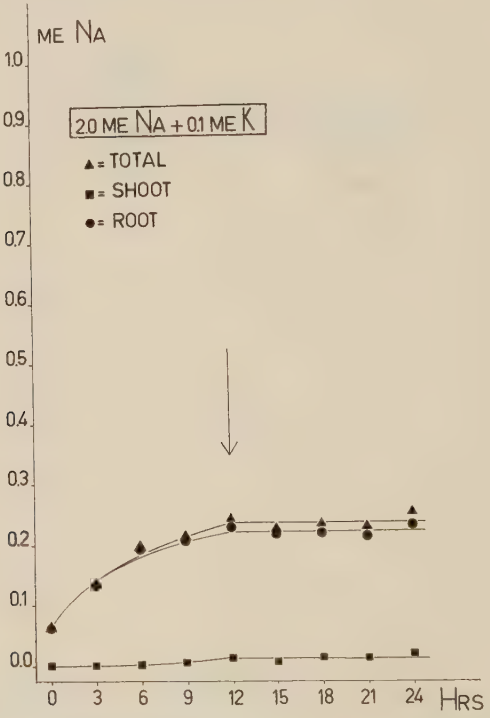

Fig. 7. Redistribution over root and shoot of Na absorbed from a solution containing 2.0 me/l Na + 0.1 me/l K after transfer of the plants to a K- and Na-free medium in relation to time.

We may account for this situation as follows (Fig. 9). All K absorbed by the specific mechanism is supposed to be transported directly to the xylem. On the other hand K taken up by the non-specific mechanism is thought to be delivered primarily to the root pool from which by some specific mechanism or other it may

reach the xylem. Both systems are supposed to operate quite independently. They may either be present in all cells of the root or each of them may be restricted to a special root zone.

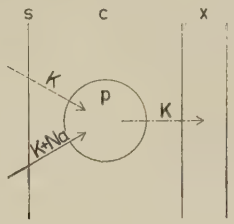

Fig. 8. Explanation in text; s = root surface, c = root cortex, x = xylem, p = root pool, ———— = non-specific absorption of K and Na, — — — — = specific absorption of K, —.—.—.—. = specific transport of K.

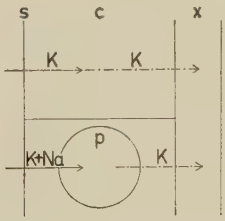

Fig. 9. Explanations in text (for denotations see Fig. 8).

There are two reasons why the operation of two such independent systems is not likely. In the first place in this picture the remarkable linearity of overall K-absorption with time is the purely accidental result of the summation of the slightly concave curve for total specific K-absorption (Fig. 3) and the unknown relation between total non-specific K-absorption and time. It should be noted though that in itself a convex course for this relation, as in the case of Na, is not at all unlikely. In the second place it has been shown (Fig. 6) that transfer of specifically absorbed K to the aerial parts continues at a decreasing rate after transfer of the plants to a K- and Na-free medium. If the picture of Fig. 9 represents the true state of affairs, the steady rate of transport of K to the shoot after absorption from a 2 me/l K-solution and subsequent transfer of the plants to a K-free medium (Fig. 5) must be the algebraic sum of the decreasing rate mentioned above and the unknown but consequently increasing rate of transfer of K absorbed non-speci-

fically. Apart from the fact that such an increase is unlikely in itself, because in the picture of Fig. 9 this transfer is supposed to occur from a pool of slowly decreasing K-content, a close comparison of Figs. 5 and 6 reveals that essentially no non-specifically absorbed K would be transported to the shoot during the first hours after the transfer to the K-free medium. It cannot be seen why this should be the case.

The preceding argument rests for the greater part on the true course of the curves of Figs. 5 and 6. Therefore it seemed useful to plot the K-content of the shoots after transfer to the K-free

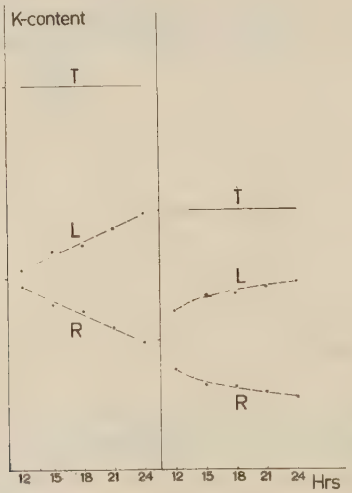

Fig. 10. Redistribution over root and shoot of K absorbed from a 2.0-me/l K-solution (left) and from a mixture of 2.0 me/l Na + 0.1 me/l K (right) after transfer of the plants to K- and Na-free media in relation to time. The K-contents of leaves (L) and roots (R) have been plotted as percentages of the total K-content (T).

media in another way, namely as fraction of the respective total K-content (Fig. 10), thus eliminating variations due to a different absorption capacity of the plant units used. The resulting curves are identical with those drawn in Figs. 5 and 6.

The alternative way in which we may account for the specific transfer to the shoot of non-specifically absorbed K is that the specific pathway involved can be used by K absorbed by either

mechanism. Fig. 11 gives a schematic representation of this situation.

It should be emphasized here that nothing is known about the actual mechanism of transport of ions from the absorption sites to the xylem. However, the high degree of specificity established in the experiments described seems to eliminate simple diffusion unless it should be based on improbably large differences in mobility or permeability between the ions inside the tissue. Therefore we must assume that during transfer from the absorption sites to the xylem K is bound to a specific carrier. Whether this "translocation carrier" is identical with the K-specific "absorption carrier" remains uncertain, but is not crucial.

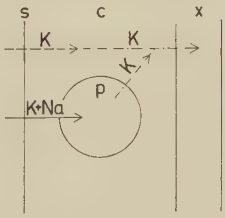

Fig. 11. Explanation in text (for denotations see Fig. 8).

Furthermore, the scheme of Fig. 11 assumes that non-specifically absorbed K is transported to the xylem via the root pool. This assumption is based on the fact that the transfer of K to the shoot continues at a steady rate when the outside supply of K is stopped (Fig. 5). Such would not be the case if a specific system with a strong binding power for K took over the ion from the non-specific mechanism on its way to the root pool. On the other hand, the actual mechanism by which K is transferred from the root pool to the xylem remains uncertain. The specific transporting system may take up K from the pool, but K may leak out as well to be picked up subsequently in the surrounding plasma. The last picture has the advantage of offering at the same time an attractive explanation of the decreasing rate of Na absorption with time (Figs. 1 and 4). For as the root pool fills with Na, part of the capacity of the non-specific absorption mechanism will be taken up for retransfer of leaking Na to the pool. On the other hand leaking K will not depress this capacity because it is bound specif-

ically and transported to the xylem. Such a situation may well underlie the remarkable linearity of overall K absorption in contrast to overall Na absorption (*cf* Figs. 1 and 2).

Summarizing, we come to the following explanation of our results (Fig. 11). K taken up by the specific absorption mechanism is transferred directly to the xylem by a K-specific transporting system. The "transporting carrier" may or may not be identical with the "absorption carrier". On the other hand K and Na taken up by the non-specific mechanism are delivered primarily to a root pool. The K-specific transporting system either removes K directly from the pool or it picks up K leaking from the pool. In the last case Na leaks as well but is returned to the pool by the non-specific mechanism. Leakage is thought to occur into but not necessarily out of the protoplasm.

Identification of the root pool with the cell vacuole and location of the K-specific transporting system in the symplast leads to a considerable agreement between our picture and the ideas of Arisz [1] on the uptake and transport of ions in Vallisneria leaves. At the same time it is consistent with the data of Broyer [3] on Br-absorption and translocation and with some more recent results of Fried *et al.* [5] concerning absorption and translocation of Rb in the same material. It should be emphasized though that the work of neither of these authors excludes the existence of separate accumulation and translocation zones in the root.

Some uncertainties remain resulting mainly from our lack of knowledge about the actual transport mechanism in the symplast. The cause of the slow increase in the rate of specific K-absorption during the first 9 hours (Fig. 3) is not clear. Unless the linearity of overall K-absorption with time (Fig. 2) should be pure coincidence, as discussed above, the specific component of this straight line does not show the same concavity. However, we may not see through these and similar details before more insight has been obtained in the actual way in which ions move to the xylem following absorption.

In the earlier publication (Bange [2]) it could not be established with certainty whether the preference for K of the specific mechanism of K-uptake was so absolute that it did not transport any Na at all. A comparison of Figs. 1 and 4 shows that the initial rate of Na-absorption is somewhat lower in the presence of 0.1 me/l K.

The effect is larger than can be attributed to competition of this small amount of K for the non-specific absorption mechanism. Apparently the mechanism may transport Na in the absence of K but in contrast to K this Na does not reach the xylem (*cf* Figs. 1 and 4), being perhaps too loosely bound to resist transfer to the vacuole by the non-specific mechanism. How Na finally does reach the xylem remains uncertain.

The fact that in the earlier work a lower value of V_{max} was found in case the non-specific mechanism transported Na is clearly due to the circumstance that the rate of Na-absorption decreased with time to reach a lower and almost steady value after about 18 hours (Figs. 1 and 4). So there is no reason to assume a different rate of breakdown of the respective ion-carrier complexes.

CONCLUSION

The experiments described have made it possible to establish a functional difference between the two mechanisms operative in the absorption of K by maize seedlings. The specific mechanism appears to be especially involved in the supply of K for transfer to the shoot where a high specific requirement for K is known to exist. On the other hand the non-specific mechanism seems primarily concerned with the supply of non-specific osmotic ion material to the roots. However, the division is not so absolute that K taken up by the non-specific mechanism could not be redistributed and transferred to the shoot as well. In this connection it should be stressed once more that our plants are typical low-salt plants, especially with respect to the alkali cations, as is evident from the initial contents of root and shoot plotted in the graphs of Figs. 1 and 2. The situation may change when the high specific requirement for K in the existing leaves becomes satisfied.

Considerations like these lead to the next question: does the leaf in some way or other convey its specific K-requirement to the root? This problem will be the subject of further experiments.

SUMMARY

The distribution of K and Na between root and shoot of intact maize seedlings after absorption periods of increasing length up to 24 hours was

determined in three series of experiments. In two additional series the redistribution between root and shoot of K and Na absorbed previously during a period of 12 hours was followed up during the next 12 hours. Concentrations were such that either the specific or both the specific and non-specific mechanism of K-absorption present in this tissue were operative.

A high degree of specificity in the transfer to the shoot became apparent, the amount of Na transported to the shoot within 24 hours being negligible in comparison to the amount of K transferred within the same period. For the most part this specificity appeared to be due to the fact that the specific mechanism of K-absorption is especially involved in the supply of K for transfer to the shoot. However, non-specifically absorbed K is also liable to transport to the aerial parts at a much higher rate than Na and, in contrast to Na, the translocation of K continues after the plants have been transferred to K- and Na-free media.

A scheme is proposed to explain the phenomena described.

ACKNOWLEDGEMENTS

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NODULATION STUDIES IN LEGUMES

IV. THE INFLUENCE OF INOCULUM STRAIN AND TIME OF APPLICATION OF AMMONIUM NITRATE ON SYMBIOTIC RESPONSE

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INTRODUCTION

Numerous investigations of the effects of combined nitrogen on the nodulation of legumes have demonstrated that maximum symbiotic performance is associated with growth media deficient in combined nitrogen (see Fred, Baldwin, and McCoy [6]; Wilson [28]; Nutman [18]; van Schreven [24]).

High levels of combined nitrogen have been shown to reduce nodule numbers, and inhibit nodule growth and fixation; the reduction in these quantities varying with the species of legume [1 22]; with the amount and form of combined nitrogen applied [9 28]; and with the time of its application to the host plant [25]. Stimulation of symbiosis by small amounts of combined nitrogen has been recorded by several workers (*e.g.* Giobel [10]; Fred and Wilson [7], MacConnell and Bond [12]).

Recent work would also suggest that the site of application of combined nitrogen to the host plant [24], the pH of the medium [5 16], growth temperatures [13 15], and general climatic conditions [11], may also influence the competitive assimilation of molecular and combined nitrogen by the nodulated legume.

In this paper evidence is presented of differences between various bacterial symbionts of the one host plant in both their infection and fixation responses to added combined nitrogen. A change with time in the sensitivity of a given association to com-

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bined nitrogen is reported. Examples of stimulation or injury of symbiosis by added nitrogen are described and it is suggested how these may be compounded to produce long-term effects on plant growth and nitrogen fixation.

GENERAL METHODS

The effects of combined nitrogen on the symbiosis of four legumes, purple vetch (*Vicia atropurpurea* Desf.), common vetch (*Vicia sativa* L. var. Golden Tares), cowpea (*Vigna sinensis* Endl. var. Poona) and barrel medic (*Medicago tribuloides* Desr. strain 173) were examined using the sand-culture technique previously described [4].

Ammonium nitrate was used as the source of combined nitrogen, and, in all but one experiment, was applied at sowing. Treatments were compared on the basis of the amounts of combined nitrogen initially applied to each pot and subsequently available to each developing plant. Plants were grown in natural daylight in glasshouses in Sydney (Experiments 1, 2, 3, 5) and Belfast (Experiment 4).

Washed, sterilised river sand was used for all experiments. The original moisture status of the sand was maintained with regular addition of distilled water and nitrogen-free mineral nutrients. Treatment replicates were randomly located on the glasshouse benches and the pots were spaced and the contained plants thinned so that no obvious restrictions of growth were imposed in any treatment. Further details of cultural conditions are given in descriptions of the individual experiments.

Inoculation at sowing gave initial bacterial populations well in excess of those suggested as necessary for maximum nodulation [21]. Nodule bacteria with code prefixes NA and SU were strains obtained from the School of Agriculture collection, University of Sydney; those with prefixes V are part of a collection of Irish and Australian strains held in the Botany Departments of the University of Sydney and Queen's University, Belfast.

In short-term experiments (1–3) primary-root nodulation (nodule number, size, and colour) was used to record the treatment responses. This was considered a reliable measure of symbiotic reaction to the initial levels of combined nitrogen since in the legumes studied infection of the primary root is completed in the first ten days of seedling growth – before appreciable amounts of nitrogen have been absorbed by the host plant. (Similar results on nitrogen uptake by legumes in agar-tube culture have been recorded by Gibson [9].) In long-term experiments (4 and 5) symbiotic responses are only indirectly related to the initial concentration of combined nitrogen since later infections of the lateral and minor root systems take place after a portion of the nitrogen supplement has been assimilated by the host plant. In such experiments nodule tissue production, nodule efficiency, and total nitrogen fixation in the host plant were determined to evaluate symbiotic activity. The relevance of these and other recorded quantities is discussed in an earlier paper [20].

RESULTS

A. *The influence of seasonal conditions on nodulation in the presence of combined mitrogen.*

Experiment 1: Poona cowpeas were grown in the presence of ten levels of ammonium nitrate applied at sowing. Quantities used were 0, 5, 10, 20, 35, 50, 75, 100, 125, 150 mg N per pot for the summer sowing (December 1958) and for the autumn sowing (April 1959) 0, 10, 20, 30, 40, 50, 75, 100, 125, 150 mg N per pot. Plants were inoculated with an effective Rhizobium strain SU 318. 20 plants were allowed to develop in each 7″-diameter, 4-litre pot. The three replicate pots of each treatment were harvested three weeks after germination. Nodule number and nodule weight per plant were determined (see Figure 1).

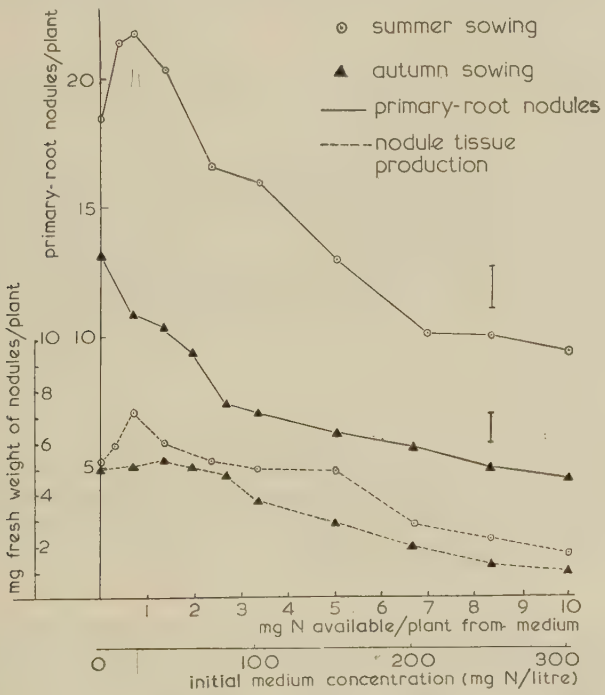

Fig. 1. Experiment 1. The effect of seasonal conditions on the nodulation response of a bacterial association of cowpea (*Vigna sinensis* Endl.) to a range of amounts of ammonium nitrate applied at sowing. Plants harvested 3 weeks after germination. 99% confidence limits are included for nodule numbers in the summer and autumn sowings.

In the favourable summer sowing, primary-root nodulation was stimulated by low concentrations of NH_4NO_3, but higher levels

progressively decreased nodule numbers. In the less favourable growth conditions of the autumn sowing no stimulation was recorded and nodule numbers were consistently lower than in comparable treatments of the summer sowing. In both seasons with moderate applications of ammonium nitrate (0 to 125 mg N/pot) any reduction in nodule numbers was wholly or partly offset by increased nodule size. This compensation was less marked and extended over a shorter range of concentrations (0 to 75 mg N/pot) in the the less favourable season. In either season the uppermost levels of added nitrogen produced unpigmented dwarf nodules with anatomical abnormalities similar to those described by Thornton and Rudorf [26].

B. *The influence of bacterial strain in nodulation response to combined nitrogen.*

Experiment 2: Barrel medic seedlings were grown in pots (6″ deep, 3″ diameter) inoculated with Rhizobium strains SU 277.1 or SU 41.237 known respectively to exhibit effective and ineffective reactions with the host plant. Ten levels of combined nitrogen (0, 2, 4, 6, 8, 12, 16, 20, 30, 40 mg N/jar) were applied as ammonium nitrate at sowing. Three replicate pots each of 15 plants were set up in each treatment. Plants were harvested in July 1959 nine weeks after germination. Primary- and lateral-root nodules were counted on each plant.

Experiment 3: *Vicia sativa* seedlings were grown in 6″ × 3″ pots at eight levels of ammonium nitrate applied at sowing (0, 5, 10, 15, 30, 50, 75, 100 mg N/pot). Eight different Rhizobium strains of varying effectiveness on *V. sativa* were used with each nitrogen treatment. Forty plants from the replicates of each treatment were examined for primary-root nodulation after four weeks growth in June, 1959.

Nodule production in Experiments 2 and 3 is recorded in Figure 2 and in Tables 1 and 2. In barrel medic primary-root nodulation with the effective strain SU 277.1 was stimulated by all but the highest levels of added nitrogen (Figure 2). A maximum in primary-root nodulation was produced by applications of 4 to 8 mg N/per pot in the ineffective association. These response patterns are markedly different from those for attendant nodulation of lateral roots. Thus, maximum number of lateral-root nodules occurred at the extremes of the nitrogen treatment range with the effective strain SU277.1). The ineffective association (SU237.41)

developed most lateral nodules with small amounts of added nitrogen, a marked depression occurring at higher concentrations. Lateral-root nodulation was much more extensive with the ineffective strain (*cf* similar results in a comparison of these two strains of Rhizobium in delayed inoculation experiments [4]).

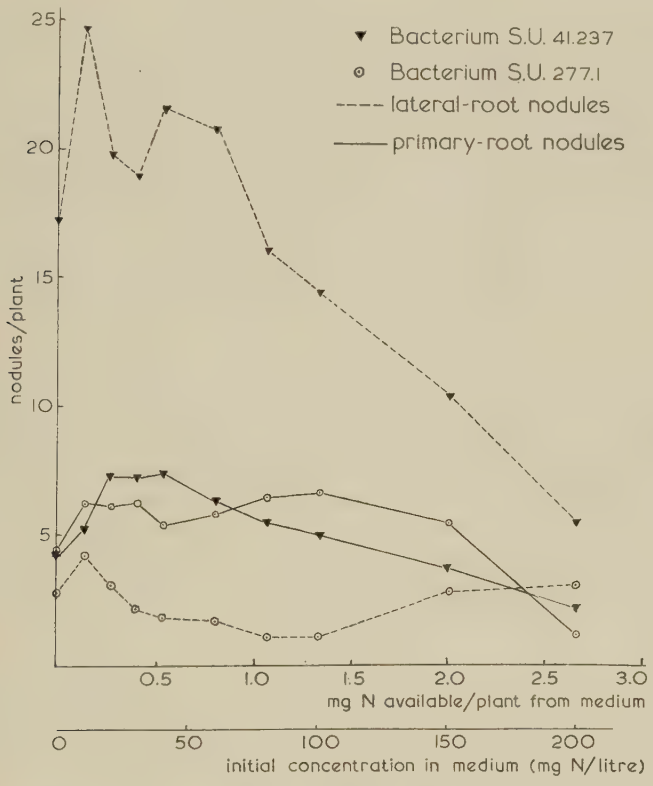

Fig. 2. Experiment 2. The influence of bacterial strain on the nodulation response of barrel medic (*Medicago tribuloides* Desr.) to a range of amounts of ammonium nitrate applied at sowing. Plants harvested 9 weeks after germination.

The eight vetch associations in Experiment 3 offer further evidence of differences in strain response to combined nitrogen (Tables 1, 2). In this experiment all strains except V.32 and SU331 exhibited stimulation of primary-root nodulation by the lower doses of combined nitrogen. The extent of primary-root nodulation

at high dose levels varied considerably between strains. For example, strains NA6, V.32 or SU331 showed more than a fifty per cent reduction in primary-root nodule numbers with 30 mg N per pot while a similar reduction in strain V.1 required a dose of 100 mg N per pot.

TABLE 1

Experiment 3: Effect of ammonium nitrate on primary-root nodulation of *Vicia sativa* L. by eight strains of Rhizobium

Treatment mg N applied/pot as NH_4NO_3	Strain							
	V1	V5	V19	V27	V32	V33	NA6	SU331
0	8.3	7.2	3.1	5.1	7.6	7.0	4.7	11.6
5	8.5	7.5	7.1	7.3	7.1	7.7	10.1	9.0
10	8.8	7.7	7.2	8.7	6.1	7.7	10.9	10.1
15	8.4	7.5	5.9	7.3	5.3	7.8	8.2	6.1
30	7.2	6.5	4.6	6.1	2.7	3.7	2.1	4.3
50	5.3	2.9	1.9	3.5	2.5	4.1	1.4	2.3
75	5.4	1.1	1.3	1.7	1.1	1.9	0.9	1.7
100	2.8	1.0	0.7	2.0	1.0	1.0	0.1	1.1
Performance* on host plant	E.I	E.I	E	E.I	E	E	I	E

* E – fully effective, E.I – moderately effective, I – ineffective.

TABLE 2

Experiment 3: Analyses of variance for primary-root nodule numbers recorded in Table 1

Source of Variation	Sum of squares	D.F.	Mean squares	*F* Ratio
Nitrogen level	5973.6	7	853.37	174.4***
Inoculum strain	876.7	7	125.24	25.6***
Interaction	1008.0	49	20.57	4.20***
Within group	4384.9	896	4.89	
Total	12243.2	959		

*** $P < 0.001$.

The origin of the six Irish strains is as follows V.1, V.19, and V.32 were isolated from vegetable legumes growing in heavily manured soils while V.5, V.27, and V.33 were isolated from native species of Vicia-inhabiting sand dune or other pioneer habitats of low nitrogen content. Although greater tolerance to added nitrogen might be expected from strains adapted to rich organic soils, no such correlation was evident from infection performances in this

experiment. There was also no apparent relationship between a strain's nodulation response to different doses of nitrogen and the type of nodule distribution produced on the host primary root.

C. *The influence of bacterial strain in fixation response to combined nitrogen.*

Experiment 4: Pots sown with purple vetch (*Vicia atropurpurea*) were inoculated with either V200, V32, V19 or SU331 strains of *Rhizobium*. Four different doses of combined nitrogen were applied at sowing to each host-bacterium combination to give supplements representing 2.5, 5.0, 7.5, or 10.0 mg N per plant as NH_4NO_3. By the end of the twelve weeks' growth period (May–July, in Belfast, Northern Ireland) all treatments had flowered and set fruit. Nodules numbers, nodule dry weights per plant, plant dry weights and plant nitrogen contents were determined for the 80 to 100 replicate plants of each of the 16 treatments.

In this and the following experiment nitrogen fixation (N_f) after a specific period of growth is calculated as

$$N_f = N_p - (N_c + N_u)$$

where N_p is the total nitrogen per plant, N_c is the nitrogen furnished by the cotyledons of the seedlings and N_u is the average amount of combined nitrogen taken up by the plant from the root medium. In both experiments analyses of the sand for ammonium or nitrate using the microdiffusion method of Bremmer and Shaw [2] indicated that more than 95% of the applied nitrogen had been taken up by the plants by the end of the growth period and N_u is, therefore, taken as being equal to the proportion of the total nitrogen available to each of the replicate plants of a treatment.

TABLE 3

Experiment 4: The influence of ammonium nitrate on nitrogen fixation in *Vicia atropurpurea* in symbiosis with four strains of nodule bacteria
Values refer to mg nitrogen fixed/plant (N_f) with standard errors. Growth period 12 weeks May–July 1960 in Belfast, N. Ireland

Mg N applied per plant	Nitrogen fixed per Plant (N_f*) Rhizobium strain			
	V19	V32	V200	SU331
0	31.2 ± 1.8	30.9 ± 2.0	32.3 ± 2.3	30.6 ± 1.7
2.5	46.2 ± 2.9	32.7 ± 5.3	37.3 ± 4.1	29.8 ± 2.3
5.0	41.6 ± 3.3	24.1 ± 3.6	25.6 ± 2.5	29.3 ± 2.9
7.5	43.0 ± 3.7	11.6 ± 4.5	26.0 ± 3.1	14.6 ± 3.7
10.0	38.5 ± 3.4	3.6 ± 2.6	21.4 ± 3.2	4.7 ± 2.6

* See text for method for calculating symbiotic fixation.

Table 3 lists nitrogen fixation in the 4 associations at the four different dose levels. There are no significant differences in fixation

between strains in the minus nitrogen series. However, with varying amounts of added ammonium nitrate the responses of the four associations are very different. Strain V.19 is very efficient at fixing nitrogen at high levels of ammonium nitrate, with marked stimulation of fixation at all dose levels. By contrast, strains V.32 and SU331 both show marked depression of nitrogen fixation with additions of 7.5 and 10 mgs of combined nitrogen.

An indication of the cause of this variation in performance at high nitrogen levels was found on examining the root systems. Primary-root nodulation in all four associations was reduced by an application of 5 mg N per plant. No visible nodules were produced on the primary- root in any of the 7.5- and 10-mg N-treatments. Lateral-root nodulation for these two latter treatments was sparse with strain V.32 and SU331, but extensive and clearly effective with strains V.19 and V.200. Symbiotic performance of purple vetch in the presence of large amounts of added combined nitrogen appears to be determined by the relative success or failure of the associated Rhizobium in infecting the lateral roots of the host plant. Presumably these later infections take place when a large fraction of the nitrogen supplement has been removed from the growth medium by the plant.

D. *The changing sensitivity of the developing association to added combined nitrogen.*

Experiment 5: Cowpea seedlings (15 plants per 7″ pot) were inoculated at sowing with an effective strain of Rhizobium (SU318). Five levels of combined nitrogen (5, 10, 20, 30, 50 mg N per pot as ammonium nitrate) were applied at five different stages in seedling growth – 0, 4, 7, 10, 14 days after germination (see Table 4 for details of seedling condition when the nitrogen was applied). A control series received no combined nitrogen.

TABLE 4

Experiment 5: Seedling condition of cowpea at time of addition of ammonium nitrate (Values recorded from sample of 15 plants harvested when application was made)

Time of application (Days after germination)	Primary root length (cm)	Plant development
4	10.0	Cotyledons emerging.
7	21.2	First leaf expanded.
10	26.6	Nodulus, small and white; first leaf pair expanding.
14	26.9	Nodules just pink, first leaf pair fully expanded.

After 6 weeks growth the three replicate pots per treatment were harvested (growth period, February–March 1959). Nodule numbers and size, plant dry weight and nitrogen content were determined.

Values for nitrogen fixation were determined as in Experiment 4. In Experiment 5 the term '*fixation benefit*' (F.B.) is introduced to compare fixation with that of control plants grown in a nitrogen free medium.

$$\text{F.B.} = N_f \text{ treatment} - N_f \text{ control.}$$

A negative value for F.B. indicates that a particular treatment has inhibited normal fixation. The success or failure of a particular nitrogen treatment can also be measured in terms of a '*fixation return*' (F.R.) defined as the increase in fixation per unit of added inorganic nitrogen.

$$\text{F.R. treatment} = \frac{\text{F.B. treatment}}{\text{N applied}}.$$

Further indications of the symbiotic status of plants may be recorded in determinations of the apparent fixation efficiency of nodule populations (mg N fixed/g dry weight nodules). Table 5 evaluates symbiotic response in each treatment. Figure 3 records fixation benefit (F.B.) values for the experiment.

Several conclusions can be drawn from the experiment.

1. A single dose of combined nitrogen gives greatest benefit when applied to 10-day old seedlings (see maxima in F.R., F.B., N_f., nodule tissue production and apparent nodule efficiency with the 10-day treatment.

2. Doses of up to 30 mg N/pot given at sowing, or at 7 or 14 days, also benefit fixation (Figure 3). The later applications (7- and 14-day treatments) give the greater stimulus to fixation.

3. A remarkable depression of symbiosis follows application at 4 days, fixation at the end of the growth period being some 10 per cent lower than in controls and as much as 50 per cent lower than treatments with comparable doses at 10 days.

4. Inhibition or stimulation of nitrogen fixation in the various treatments is very closely related to the pattern of nodule tissue production and apparent nodule efficiency (compare Columns *5*, *7*, and *8* of Table 5).

5. Primary-root nodule numbers are not significantly reduced by applications of nitrogen made at 4 days or later in seedling growth. However, applications at sowing produce the usual decrease in nodulation expected from autumn growth of the cowpea-SU318 association (see Experiment 1).

TABLE 5

Experiment 5: Symbiotic response of the cowpea — SU318 association to a timed application of ammonium nitrate
Values are means for the 40 to 50 plants harvested from each treatment 6 weeks after sowing

1 Time of application Days after sowing (see Table 4)	2 mg N applied per plant as NH_4NO_3 N_u*	3 Total** nitrogen per plant (mg N) N_p*	4 Nitrogen fixation per plant (mg N) N_f*	5 Fixation benefit per plant (mg N) F.B.*	6 Fixation return per plant F.R.*	7 Dry weight nodules per plant mg	8 Fixation efficiency (mg N fixed per g dry wt. nodules)	9 Primary root nodules per plant
Control	0	15.49	13.25	—	—	1.89	7.1	18.6
0	0.33	17.68	15.11	1.86	5.59	1.85	8.2	17.1
	0.67	16.79	13.89	0.64	0.96	1.81	7.5	16.0
	1.33	16.85	13.28	0.03	0.02	1.76	7.6	13.8
	2.00	17.60	13.36	0.11	0.06	1.80	7.4	11.9
	3.33	18.45	12.88	—0.37	—	1.73	7.5	10.3
4	.33	16.23	13.66	0.41	1.23	1.86	7.3	16.5
	.67	15.45	12.55	—0.60	—	1.82	6.9	16.6
	1.33	15.62	12.05	—1.20	—	1.82	6.6	16.8
	2.00	16.22	11.99	—1.26	—	1.78	6.7	17.1
	3.33	17.68	12.11	1.14	—	1.81	6.7	17.3
7	0.33	18.75	16.18	2.93	8.80	2.04	7.9	18.5
	0.67	17.92	15.02	1.77	2.65	2.10	7.2	18.3
	1.33	18.43	14.86	1.61	1.21	2.09	7.1	18.6
	2.00	19.69	15.45	2.20	1.10	2.09	7.4	18.7
	3.33	19.05	13.48	0.23	0.7	1.81	7.5	17.6
10	0.33	19.69	17.12	3.87	11.62	1.89	9.1	18.2
	0.67	22.12	19.22	5.97	8.95	2.12	9.1	18.5
	1.33	20.79	17.23	3.98	2.98	2.12	8.1	18.6
	2.00	21.20	16.97	3.72	1.86	2.11	8.1	18.0
	3.33	22.43	16.86	3.61	1.08	1.94	8.7	18.8
14	0.33	19.55	16.98	3.73	11.20	1.96	8.7	18.4
	0.67	20.00	17.08	3.83	5.74	2.08	8.2	18.5
	1.33	18.48	14.91	1.66	1.24	1.87	8.0	18.3
	2.00	18.16	13.92	0.67	0.36	1.81	7.7	18.7
	3.33	19.60	14.03	0.78	0.23	1.72	8.1	18.0

* Symbols defined in text.
** Includes cotyledon nitrogen (2.24 mg N/plant).

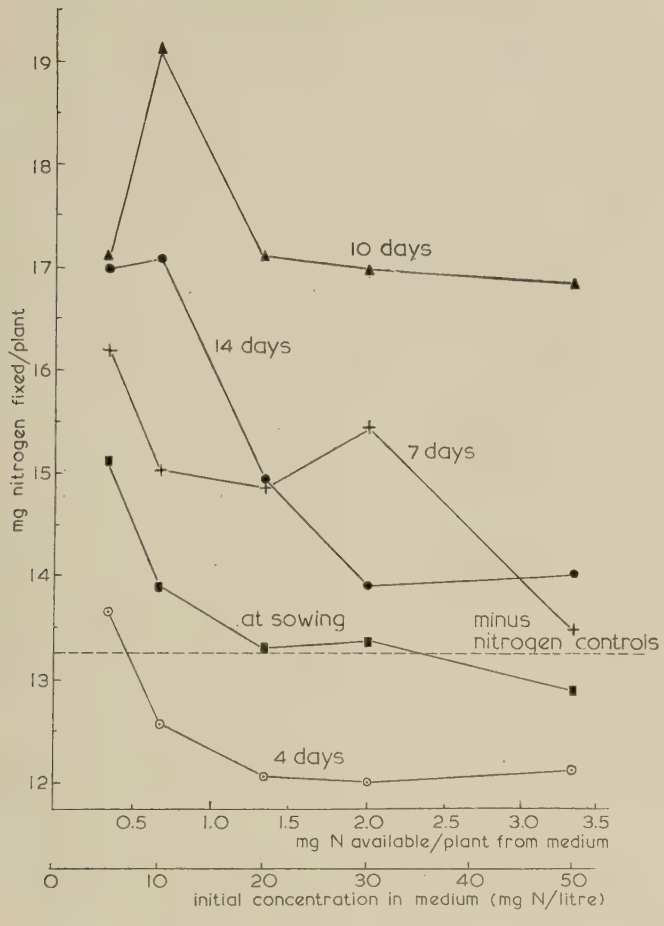

Fig. 3. Experiment 5. Symbiotic response of the cowpea-Rhizobium SU318 association to timed applications of ammonium nitrate. Values for nitrogen fixation refer to a 6-week period of growth. Complete data for the experiment are to be found in Tables 4 and 5.

DISCUSSION

Bacterial strain characteristics in symbiotic response to combined nitrogen

The experiments on barrel medic and the two species of vetch (Experiments 2, 3, 4) reveal marked differences in bacterial strain response to added ammonium nitrate. Using nodule number and

nitrogen fixation as measures of symbiotic reaction we can divide the studied host–Rhizobium pairings into two classes:

(a) those in which addition of inorganic nitrogen depresses symbiosis and

(b) those in which symbiosis is stimulated to some degree by added nitrogen. In this category it is possible to select some associations which are particularly effective in fixation even in the presence of large amounts of medium nitrogen (*e.g.* the V.19–purple vetch association Experiment 4). In this latter example excessive amounts of combined nitrogen inhibit nodulation of the primary root and symbiotic recovery takes the form of an unusually extensive and effective nodulation of later formed parts of the root system. Clearly an Experiment covering the full growth cycle of the association is needed to appreciate the full implications of bacterial strain response to combined nitrogen.

The existence of such well-defined differences between Rhizobium strains suggests the possibility of selecting nodule bacteria for the most efficient symbiosis in the soil nitrogen conditions to be encountered in a particular locality. The majority of bacterial strains used as commercial inoculants have been selected from effectivity trials in nitrogen-free media and have undoubtedly proved extremely effective in the nitrogen deficient soils where they have been introduced. However, there is no reason to assume that effectiveness in such a trial implies comparable performance in more fertile soils. The (b) type of strain might well play an important role in the maintenance of highly fertile conditions in soils or pastures regularly receiving nitrogenous fertilizers.

The complication of environment in symbiotic response must not be overlooked. In Experiment 1 the cowpea association benefited from added nitrogen in one set of growth conditions, while in another season symbiosis was restricted by any amount of added nitrogen. Similarly in recent experiments studying the interaction of light and temperature on symbiotic fixation (Pate, Nature **192**, 637 (1961)) it has been found that a particular host–bacterium relationship may exhibit full efficiency in symbiosis with one light-temperature combination while appearing to be completely ineffective in a different environmental situation. Indeed it is clear that the symbiotic responses described here, and in other publications, represent only series of cross sections of complex

interactions between symbiotic genotypes and environmental variables. It is reasonable to assume that through long-term association with a host plant in a particular climate and soil fertility condition bacterial variants might become established showing special adaptations for successful association with the host plant. Evidence for adaptations of this kind have been demonstrated by Nutman and Read [17] for old clover stands in Scandinavia. A close matching of *Rhizobium trifolii* populations to the potassium status of soil and legume has been reported by Roberts and Olsen [23] in North America. There is, of course, no reason to suspect that adaptations of this kind need necessarily involve efficiency in symbiosis. Indeed, several surveys of indigenous rhizobia of various native legumes have provided evidence that ineffective relationships with the host plant may dominate in some localities and soil conditions [6 28].

The nature and source of symbiotic inhibition or stimulation by combined nitrogen

Numerous theories have been proposed to interpret the effects of combined nitrogen on legume symbiosis. The carbohydrate–nitrogen hypothesis, as proposed by Wilson [28] is consistent with behaviour over a range of environmental conditions, but provides no adequate physiological explanations for response to combined nitrogen.

Restriction of symbiosis by combined nitrogen has been ascribed to effects on nodule structure (Thornton and Rudorf [26]), to decreased sugar levels in the cell sap, (Orcutt and Wilson [19]) or to the indirect effects of added nitrogen tying up the plant carbohydrate, with attendant reduction in the plant rhizosphere excretion of bacterial infection factors (Richardson *et al.* [22]). Stimulation of symbiosis by small amounts of added nitrogen has been ascribed to more rapid root growth, and hence the production of more infection sites (Richardson *et al.* [22]) and to the indirect effect of nitrogen benefiting early seedling growth [10].

Interpretations of the depression of symbiosis by combined nitrogen are conflicting. Some experiments, using vertically divided root culture indicate a localised action at the root surface. Here, nodulation was shown to be inhibited on the part of the root system exposed to combined nitrogen, but was unaffected on the part

growing in nitrogen-free conditions [8 27]. Other experiments would indicate a more widespread internal action of combined nitrogen. For example leaf sprays of urea (van Schreven [24]) and ammonium nitrate (Dart and Pate, unpublished data) can reduce nodulation. Similarly, pre-treatment of the roots with combined nitrogen before inoculation [12], and nitrogen applications to the lower half of horizontally-divided root cultures [3] have also been shown to inhibit symbiosis.

Much of the confusion about the effects of combined nitrogen on symbiosis has undoubtedly arisen from an attempt to interpret results without full consideration of the time or season of application of the inorganic nitrogen or the site of its action in the host plant. The experiment on timed nitrogen additions to cowpea (Experiment 5) illustrates how the sensitivity of the cowpea association to combined nitrogen can change as symbiosis is established in the seedling. Several sites of action may be involved in this pattern of response each a possible source of benefit or injury to subsequent symbiosis.

(i) Nitrogen applied at sowing. Under suitable climatic conditions small supplements of inorganic nitrogen at sowing may benefit symbiosis by increasing seedling growth rate and the number, size and efficiency of the nodule complement. This enhanced symbiotic potential may be just a corollary of increased seedling vigour. Excessive amounts of nitrogen may depress nodulation by reducing the number of infection sites and/or the number of successful infections of the primary roots. The latter damage may not be permanent since slight reductions in nodule numbers may be offset by increases in nodule size and efficiency (see also Experiment 1). Similarly an even more severe restriction of early nodulation may be offset by a more extensive and effective nodulation of the lateral root system (see Experiment 4).

(ii) Nitrogen applied as the cotyledons unfold. The developing association is particularly sensitive to applied nitrogen at this stage. Possibly a channelling of photosynthetic products into protein synthesis in the expanding shoot system may limit the amount of carbohydrate and other growth materials available for the root system and its developing nodules. Nodule numbers

are not affected by combined-nitrogen applications at this or later stages in host growth indicating that once nodule development has proceeded beyond a certain stage it will continue even in the presence of large amounts of medium nitrogen. However, subsequent growth and fixation of these nodules is severely retarded.

(iii) Nitrogen applied as the first nodules appear on the roots. After nodule initiation the seedling often goes through a period of so-called 'nitrogen hunger' before the nodules are sufficiently developed to supply the increasing nitrogen demands of the host plant. At this stage all growth activities of the plant benefit from added nitrogen and, in particular, the symbiotic system exhibits its greatest response to combined nitrogen. The higher fixation efficiencies recorded from nitrogen treatments at this stage suggests that part of the added combined nitrogen may be utilised in producing a more effective organisation of bacteroid tissue in the developing nodule.

(iv) Nitrogen applied as the first nodules become pigmented. An application of inorganic nitrogen at the onset of fixation does not appear to have as favourable an effect on symbiotic development as it does when made during the 'nitrogen hunger' stage of seedling development. Nodule growth and fixation are probably restricted by competition for available carbohydrate with the processes involved in the assimilation of the applied combined nitrogen.

The possible use of nitrogenous fertilisers as a means of improving symbiotic yield under field conditions

The principle underlying the use of nitrogenous fertilisers to increase the yield of nodulated legumes rests on the well-known fact that plant growth coupled to even the most efficient symbiosis cannot match that possible when the host plant has access to optimum amounts of inorganic nitrogen. The pot trials described in the present paper illustrate how a nitrogenous fertiliser applied in early seedling establishment can act as an *investment* producing a substantial return in additional fixed nitrogen in the subsequent life cycle of an association. An investment of this kind clearly acts by setting a higher growth rate and nodulation potential

in the young symbiotic association which, if given correct conditions in later development, will provide cumulative benefits to plant yield and nitrogen fixation. The procedure for obtaining such a stimulus to fixation would involve the following considerations:

(i) The correct timing and quantity of application of a nitrogenous supplement should be specified as shown in Experiment 5. The finesse of timing an application consists of making the bulk of the nitrogen applied available to the plant during the nitrogen-hunger period immediately following the exhaustion of cotyledon nitrogen reserves. When a moderate quantity of nitrogen is applied at this time, nodule and plant growth are benefited without disturbing the pattern of further infection and root development, and the major portion of the supplement is utilised in growth processes before there is any chance of unassimilated nitrogen interfering with the early fixation of the nodules.

The timing of nitrogenous supplements might prove difficult in field conditions where weather conditions and leaching from the soil might complicate the issue.

(ii) In conditions where artificial inoculation can be practised, bacterial strains of proven symbiotic tolerance of inorganic nitrogen might be selected with special reference to the particular host plant to be grown and its predicted climate and fertiliser treatments. Experiment 5 illustrates how long-term pot trials could be used for such strain selections.

SUMMARY

The influence of combined nitrogen (as ammonium nitrate) on the symbiotic performances of selected bacterial associations of four legumes was examined using sand culture.

In barrel medic (*Medicago tribuloides* Desr.) and vetch (*Vicia sativa* L. and *V. atropurpurea* Desf.) bacterial partnerships of a host plant varied greatly in their nodulation responses to a range of amounts of nitrogen applied at sowing. Some bacterial strains exhibited varying degrees of stimulation of nodule number, growth and fixation by low or medium amounts of nitrogen. Higher levels of combined nitrogen depressed symbiosis. Other strain responses showed a severe restriction of symbiosis with any amount of added nitrogen.

Seasonal influences conditioned symbiotic responses to combined nitrogen in an association of cowpea (*Vigna sinensis* End.). With a summer sowing small amounts of ammonium nitrate added at sowing benefited later sym-

biotic development. No such stimulation was evident in an autumn sowing and symbiotic injury from high levels of nitrogen was greater than in the summer sowing.

The developing association of cowpea was found to be most sensitive to ammonium nitrate added just as the first leaves unfolded. Here damage was manifest in a permanent elevation of the top: root ratio with subnormal growth and functioning of nodules. Greatest benefit from added inorganic nitrogen followed applications made as the first nodules appeared on the primary root. In this case added combined nitrogen acted as an investment providing returns in additional fixation equivalent to 5–10 times the amount of nitrogen originally fed to the seedling and representing some 50 per cent greater total fixation than in minus-nitrogen controls.

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PHOSPHORUS TRANSFORMATIONS DURING PLANT DECOMPOSITION

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The object of the following work was to follow the transformations undergone by plant phosphorus (a high proportion of which is inorganic) during decomposition in order to gain further insight into the conditions governing the nature and amount of the residual phosphate compounds reaching the soil. The problem is simplified by the fact that with most plant materials the initial supply of inorganic phosphorus is adequate for both the metabolic and synthetic processes of the microbial population throughout decomposition. Changes in microbial phosphorus during the development and decline of the population are therefore largely involved rather than mineralisation of plant organic compounds, which occurs at a slow rate. A difference may therefore be drawn between organic nitrogen and organic phosphorus with regard to decomposition and mineralisation. The amount of inorganic nitrogen in plant material is usually small so that mineralisation of organic nitrogen (which readily occurs with most plant materials) is essential for the decomposition process. On the other hand supplies of inorganic phosphorus are usually adequate for microbial needs during decomposition and they are not dependent on the mineralisation of organic phosphorus. The important factor in plant decomposition-phosphorus relationships is the extent to which the inorganic phosphorus initially present is converted to microbial organic phosphorus and how far this is recoverable (through dephosphorylating processes) as inorganic phosphorus on further decomposition or when decomposition is virtually complete.

The decomposition of various plant materials was followed in the macro-respirometer (Birch and Friend [4]) with reference

to changes in the various fractions detailed later. The results indicated that further experiments using glucose (instead of plant material) and various amounts of inorganic nitrogen and phosphorus salts should be carried out. The relevant part of this study is mentioned in the present paper.

EXPERIMENTAL

Materials

Finely ground (20 mesh) dry plant materials were selected which covered a range of phosphorus contents (Table 1).

TABLE 1

Samples used in the experiments

Material	Lab. No.	% N	A % P (total)	B % P (inorganic)	$\frac{B \times 100}{A}$	Group
Kikuyo grass . . .	33	2.92	0.228	0.196	86.0	1
Sorghum leaf . . .	756	3.07	0.348	0.273	78.0	Young
Rhodes grass. . . .	35	2.42	0.192	0.172	87.0	
Star grass.	2436	1.60	0.081	0.050	62.0	
,, ,, ...	2449	1.78	0.171	0.086	50.0	2
,, ,, ...	2457	1.40	0.181	0.085	47.0	Mature
Sorghum leaf . . .	745	3.84	0.532	0.323	61.0	

Methods

Four hundred milligrams of plant material were added to 50 g of fine (40–100 mesh) acid-washed, phosphorus-free sand in an 8-oz. wide-mouth sample bottle. Six millilitres of water were then added and the bottle was connected to the remainder of the respirometer unit and incubated at 25°C. A solution of sodium hydroxide was included in the bottle to trap evolved carbon dioxide. Twelve replicates were prepared in this way. After two days decomposition one of the bottles was withdrawn and the contents analysed. Other replicates were taken after 3, 4, 5, and 6 days decomposition and subsequently at longer intervals. An additional replicate was prepared for analysis immediately after adding the water (zero decomposition time).

On removal of the sample the amount of carbon mineralised was determined titrimetrically (Birch [3]). The sample was then divided into two. One half was placed immediately while moist in a vacuum desiccator containing chloroform, and left under reduced pressure in contact with the vapour for 24 hours. The other half was divided into four equal parts. One of these (A) was shaken with 25 ml of water in a wrist-action shaker for 1 hour and another (B) with 25 ml of *N* sulphuric acid. The third portion (C) was refluxed for 1 hour with 25 ml *N* sulphuric acid while the fourth portion

(D) was treated with 25 ml of *N* sulphuric acid and kept at 100°C in a sealed tube for 5 days.

After the foregoing extractions (A), (B), and (C) were filtered and an aliquot (2.5 or 5.0 ml depending on the plant material) added to 2 ml of Truog's [14] reagent diluted with about 30 ml of water (to avoid contact of the aliquot with strong acid with possible hydrolyses of any organic phosphorus extracted under (A) or (B)) in a 50-ml graduated flask. After making to the mark with water the phosphorus content was determined photometrically 14 minutes after the addition of 5 drops of 1% stannous chloride solution in 2 *N* hydrochloric acid. The aliquots from treatment (B) and (C) were neutralised with 1 : 1 ammonia solution, using a 0.5% aqueous solution of *p*-nitrophenol as indicator, before adding to Truog's reagent. With treatment (D) 3-ml aliquots were withdrawn after 5 days and phosphorus determined as for (B) and (C). The chloroform-treated samples were similarly divided into four parts and put through the same extraction procedures. The complete procedure described above was applied to some, but not all, of the samples.

Nature of the phosphorus fractions

(A) Water extracts inorganic phosphorus. At zero decomposition time it gives the amount of inorganic phosphorus in the plant material.The difference between this amount and that subsequently extracted after different periods of decomposition is equivalent to the organic phosphorus content of the micro-organisms (Caldwell and Hinshelwood [8]).

(B) Cold acid extracts inorganic phosphorus and, in addition, adsorbed inorganic phosphorus in the microbial cells. Caldwell [7] estimated that only about 10 per cent of bacterial phosphorus was present in inorganic form in the cells of *Bact. lactis aerogenes*. In the present studies the results obtained with cold-water and cold-acid extraction were often similar. Reference to the data for cold-acid extractions is therefore subsequently made only when there was a divergence in values between the two methods. This may be due to hydrolysis of glucose-1-phosphate which, according to Anderson [1] is hydrolysed by mild acid treatment.

(C) The amount of inorganic phosphorus obtained after refluxing 1 hour with boiling *N* sulphuric acid minus the amount of water-soluble inorganic phosphorus measures the amount of acid-labile phosphorus. The components of this fraction were not determined but according to Caldwell and Hinshelwood [8] they probably consist of ribose-3 phosphate, adenosine-3 phosphate and guanosine-3 phosphate. These are phosphorylated metabolic intermediates.

(D) Treatment of the samples for five days at 100°C with *N* sulphuric acid resulted in the almost complete hydrolysis of all the microbial organic phosphorus (including acid-labile) to inorganic phosphorus. The amount of acid-stable phosphorus may therefore be obtained by the difference between organic phosphorus and acid-labile phosphorus. Included in this group are phospholipoids and nucleic acids (Caldwell [7]).

Treatment of the samples with chloroform vapour prior to extraction measures changes in enzyme activity during decomposition since bacteria, which were found to be mainly operative throughout decomposition, are susceptible to this treatment. Enzymatic activity following death in this way is particularly marked during the early stages of decomposition, especially with the Group-1 samples, and brings about the hydrolysis of both acid-labile and acid-stable compounds to inorganic phosphorus. The chloroform experiments also appear to distinguish between enzyme systems responsible for the hydrolysis of both acid-labile and acid-stable organic phosphorus to inorganic phosphorus and for the conversion of acid-stable to acid-labile phosphorus. Further reference to this aspect of the work is made in Expt. 4.

Experiment 1. Changes in the amount of inorganic phosphorus during decomposition

Changes in water-soluble inorganic phosphorus for 7 samples are shown (smoothed out) in Fig. 1. Three patterns are evident

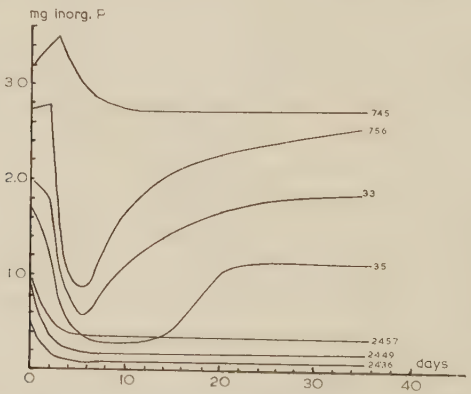

Fig. 1. Uptake and release of inorganic P during decomposition of various plant materials (1 g).

namely (1) almost complete recovery of the inorganic phosphorus initially utilised by the micro-organisms, (2) partial recovery, and (3) no recovery. These patterns were duplicated with glucose and inorganic salts (Expt. 5). The course of carbon mineralisation is shown in Fig. 2 where curve *A* is typical of plant materials

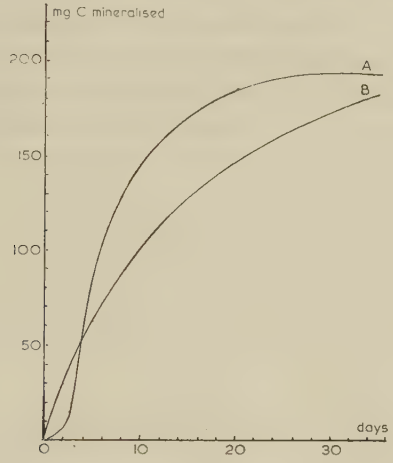

Fig. 2. Course of organic carbon mineralisation during decomposition of young (curve A) and mature (curve B) plant materials (1 g).

(Group 1, Table 1) giving a complete or partial recovery of inorganic phosphorus and curve *B* is typical of those giving no recovery (Group 2, Table 1). Group-1 samples show little decomposition during the first two days. Thereafter there is a marked increase in decomposition, the rate subsequently falling to a very low level. Group 2 exhibit a more uniform rate of decomposition from the start. The magnitudes of decomposition do not, in general, differ greatly but Group-1 samples generally decompose to a greater extent than the Group-2 samples over the 5 week period. It appears that the difference between decomposition curves *A* and *B* is due to differences in substrate availability rather than to microbiological differences since similar bacterial populations were involved in all instances throughout decomposition.

The loss of structural identity and build-up of ammonia during decomposition of the Group-1 samples (behaviour not shown by Group 2) indicates that Group 1 is concerned with young plant

material (supplying readily utilisable substrate) and Group 2 with mature material the components of which are more resistant to decomposition which is, therefore, more prolonged.

A feature of interest in Fig. 1 is the curve for Sample 745. In spite of the relatively high organic phosphorus content of this sample there is no evidence of mineralisation of plant organic phosphorus after 5 weeks and the form of the curve is not dissimilar to those for the remaining Group-2 samples of much lower phosphorus content. The inorganic phosphorus status during decomposition appears to be more closely related to substrate availability than the amount of organic phosphorus initially present in the plant.

Experiment 2. The effect of chloroform vapour on the amount of inorganic phosphorus extracted

Samples withdrawn at various stages of decomposition were halved. One half was extracted immediately with water and the other half after 24 hours exposure to chloroform vapour. In this way the amount of microbial

TABLE 2

Hydrolytic effect on microbial organic phosphorus after killing the cells with chloroform

Decomposition period (days)	Sample							
	33		756		49/57 †		2457	
	O.P.*	% Rec.**	O.P.	% Rec.	O.P.	% Rec.	O.P.	% Rec.
2	0.132	Nil			0.702	58	0.320	28
3	1.245	79	0.924	58	0.649	53	0.333	35
4	1.351	84	2.540	70	0.636	35	0.384	23
5	1.324	76	2.001	90	0.834	49	0.384	23
6	1.086	63	2.052	80	0.702	25		
7	1.324	48			0.768	34		
9			1.462	20	0.768	45	0.538	19
10			1.591	11	0.795	43		
12							0.474	8
16	0.556	Nil			0.689	6		
17			0.821	4			0.538	12
20	0.741	21	0.949	0			0.513	20
21					0.874	26		
25			0.949	0			0.589	17
26	0.586	18						
27					0.795	13		
31			1.155	0			0.437	6
37	0.238	Nil			0.662	0		

* O.P. = mg microbial P based on 1 g plant material taken initially.

** % Rec. = percentage of O.P. hydrolysed to water-sol. inorg. P after killing the cells.

† 49/57 = mixture of Samples 2449 (2 parts) and 2457 (1 part).

organic phosphorus enzymatically hydrolysed to inorganic phosphorus after killing the cells can be calculated by difference. Table 2 shows the results for two Group-1 samples and two Group-2 samples.

The data in Table 2 may be considered in conjunction with the effect of chloroform shown diagrammatically in Figs. 3, 4, and 5. During the early stages of decomposition a large viable population exhibiting high enzyme activity is operating. Death of the young population (through chloroform) causes reversible reactions with enzymatic dephosphorylation of organic to inorganic phosphorus. The sudden drop in enzyme activity shown by Samples 33 and 756 more or less coincides with the end of the active decomposition period. At this stage the cells are senile, the enzymes decay, and the chloroform effect is small. Continuity of enzyme activity depends on the production of new cells, and the less dramatic decline in the effect of chloroform on dephosphorylation with Samples 2457 and 49/57 indicates the continued presence of new cells because of the more prolonged availability of substrate obtaining with these samples.

It should be mentioned here that plant material moistened and immediately exposed to chloroform vapour always gives a slightly greater amount of water-soluble inorganic phosphorus than non-exposed samples. This may be due to plant enzyme activity and/or diffusion of inorganic phosphorus from the plant during the 24 hours exposure to chloroform. The magnitude of the chloroform effect for zero decomposition time has, however, been subtracted from the magnitude of the effect for subsequent decomposition periods in arriving at the data given in Table 2, which, therefore, refers entirely to the effect of chloroform on bacteria. Should the chloroform effect for zero decomposition time not be operative once decomposition starts the percentage recoveries in Table 2 would be greater throughout particularly with Samples 49/57 and 2457 where the correction is applied to smaller absolute values for the chloroform effect then with Samples 33 and 756, thereby leading to a more marked reduction in the precentage-recovery data.

The amount of inorganic phosphorus released following chloroform treatment may, on the basis of the foregoing experiment, find wider application as an index for the presence of viable bacterial cells.

Experiment 3. Effect of prolonged decomposition on the recovery of inorganic phosphorus

In this experiment 6 of the samples listed in Table 1 were incubated for 3 months, and the amounts of inorganic phosphorus determined by water extraction before and after decomposition (no intermediate determinations were made). The results are given in Table 3. The slight differences between the values in column 3 (Table 3) and column 5 (Table 1) are attributed to sampling errors and differences in vibration rates during extraction.

TABLE 3

Recoveries of inorganic P after 3 months decomposition. (On the basis of 1.0 g of plant material initially taken)

Sample No.	mg C mineralised	Inorganic P, mg	
		Before decomp.	After 3 mths. decomp.
756	294	2.754	2.448
35	292	1.648	0.761
33	238	1.929	1.697
745	222	3.372	3.582
2436	204	0.504	0.026
2457	205	1.028	0.437

There is no evidence that even on prolonged decomposition plant organic phosphorus is mineralised to give greater amounts of inorganic phosphorus than those initially present, though this must occur during the later slow process of humus decomposition.

Experiment 4. Interrelationships between the various phosphate fractions during decomposition

Changes in the amounts of the various fractions described at the start of the experimental section were followed with Sample 756 (Group 1) and 2457 (Group 2). Sample 33 (Group 1) was also studied with the exception of the 5-day hydrolytic period with *N* sulphuric acid at 100°C. The results obtained for this period with Sample 756 are, however, applicable in interpreting the results with Sample 33.

Sample 756. During the first 2 days there was a small increase in the amount of inorganic phosphorus which together with the main supply was subsequently utilised by the bacteria when decomposition started. Day 2 is therefore taken as the starting point in this instance. The amounts of acid-labile, acid-stable,

and chloroform-labile phosphorus were corrected throughout to allow for the amounts initially present in the undecomposed material.

The data are plotted in Fig. 3 to show the amount of bacterial organic phosphorus (Graph 1) present during the various stages of decomposition (assuming 1 g of sample initially taken). The amount of acid-labile phosphorus is shown by Graph 2. The difference between Graphs 1 and 2 represents acid-stable phosphorus. The amount of phosphorus hydrolysed after 5 days with *N* sulphuric acid at 100°C corresponded almost exactly, throughout decomposition, with the bacterial organic-phosphorus content. Since it is therefore, practically synonymous with Graph 1 it is not shown in the figure. The broken lines (Graphs 4 and 5) show the amount of inorganic phosphorus obtained when the samples were exposed to chloroform vapour prior to extraction with water and hot *N* sulphuric acid (1 hour) respectively.

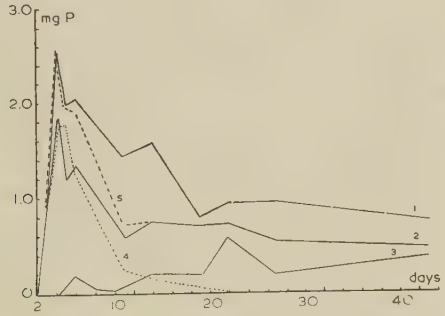

Fig. 3. Sample 756. P-transformations during decomposition. Graph 1, microbial organic P. This is made up of acid-labile P (Graph 2) and acid-stable P (Graph 1–Graph 2). Cold-acid-extractable P (Graph 3) is part of the acid-labile fraction. Graphs 4 and 5 show the amounts of inorganic P recovered from the organic P when the samples are exposed to chloroform vapour prior to extraction with water (Graph 4) and hot acid (Graph 5).

Fig. 3 shows that an initial rapid build-up of organic phosphorus is followed by a decline as dephosphorylation to inorganic phosphorus takes place. The close parallelism between Graphs 1 and 2 during the first 12 days indicates that dephosphorylation largely involves hydrolysis of the acid-labile organic phosphorus compounds. On the twelfth day a change in the dephosphorylation process

takes place and inorganic phosphorus starts to be formed at the expense of the acid-stable organic phosphorus, while the amount of acid-labile phosphorus declines slowly. Hydrolysis of the acid-stable forms may be due to lysis of the cells formed earlier as a result of which contact is established between dephosphorylating enzymes and acid stable compounds formerly separated from these. An alternative possibility is mentioned in the discussion. By the 17th day practically all the organic phosphorus is in the acid-labile form. Thereafter there is apparently some conversion of acid-labile to acid-stable phosphorus after which the latter slowly mineralises. By the 41st day the residual organic phosphorus contained approximately 65 per cent acid-labile phosphorus and 35 per cent acid-stable phosphorus. The amount of cold-acid-extractable phosphorus is negligible during the early stages of decomposition but shows a tendency to increase during the later stages.

Treatment of the sample with chloroform vapour prior to extraction with water resulted during days 2 to 6 in the recovery of inorganic phosphorus in amounts almost equal to the acid-labile values (compare Graphs 2 and 4) after which the effect of chloroform declines noticeably. When the chloroform-treated samples were hydrolysed for 1 hour with boiling *N* sulphuric acid the amount of inorganic phosphorus recovered was greater than that from the non-chloroform treated samples (*cf* Graphs 5 and 2) and between the 2nd and 6th day accounts for practically all the bacterial organic phosphorus. The effect was negligible by the ninth day. Enzyme activity following chloroform treatment during the early stages converted acid-stable to acid-labile phosphorus. There is therefore a period during the early stages of decomposition when enzyme activity following chloroform treatment can convert acid-labile to inorganic phosphorus, and acid-stable to acid-labile phosphorus. Conversion of acid-stable to inorganic phosphorus can also occur as shown with sample 33.

Sample 33. This sample, after decomposition, gave an almost complete recovery as inorganic phosphorus of the bacterial organic phosphorus initially formed. The amounts of the various fractions present at progressive stages of decomposition are shown in Fig. 4.

The overall changes during the early stages of decomposition are similar to those for Sample 756. An initial rapid build-up of

organic phosphorus (Graph 1) is followed by its dephosphorylation involving first the conversion of acid-labile forms (Graph 2) and then of acid-stable forms, (Graph 1 minus Graph 2) to inorganic phosphorus. A marked decline in acid-stable phosphorus starts only on the 20th day. From the 16th to the 26th day there is an increase in acid-labile phosphorus when secondary decomposition may occur resulting in renewed bacterial synthetic processes. Thereafter this again declines giving rise to inorganic phosphorus the amount of which is further enhanced by steady dephosphorylation of acid-stable phosphorus (Graph 1 minus Graph 2). The final composition of the residual organic phosphorus, namely about 75 per cent acid-labile and 25 per cent acid-stable is similar to that for sample 756.

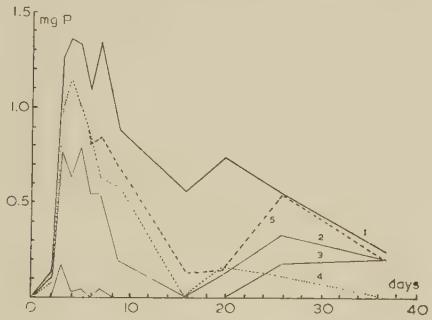

Fig. 4. Sample 33. Gramphs 1 to 5 as under Fig. 3. Graphs 4 and 5 are synonymous up to day 6.

The amount of cold-acid extractable phosphorus (Graph 3) is negligible until the 20th day after which, as with Sample 756 there is an increase until, at the end of decomposition, the amount is equal to acid-labile phosphorus. This reflects the presence of easily hydrolysable organic phosphorus compounds (possibly glucose-1-phosphate) rather than microbially adsorbed inorganic phosphorus (see under Experimental) since it is unlikely that about 75 per cent of bacterial organic phosphorus would be inorganic. The whole of the acid-labile phosphorus with sample 33, and part of it with Sample 756, is therefore in a readily hydrolysable form when decomposition is virtually complete.

Graphs 4 and 5 refer to samples exposed to chloroform vapour prior to extraction. Graph 4 shows the amount of inorganic phos-

phorus extracted with water and Graph 5 the amount produced after hydrolysis with *N* sulphuric acid at 100°C for 1 hour. Graphs 4 and 5 for the first 6 days are almost identical and show that chloroform treatment has effected the enzymatic hydrolysis of both acid-stable and acid-labile phosphorus to inorganic phosphorus, and that a considerable proportion of the organic phosphorus is susceptible to this treatment.

After the 6th day enzymatic activity following chloroform treatment becomes increasingly ineffective and is negligible by the 16th day. After the 20th day there is a recovery in enzyme activity involving the conversion of acid-stable to acid-labile forms. This may reflect secondary decomposition (consequent upon lysis of bacterial cells produced earlier) which would give rise to a newly developing population susceptible to exposure to chloroform and containing active enzyme systems.

Sample 2457. This sample gave little recovery of inorganic phosphorus even after three months decomposition (see Table 3). The distribution of the various fractions during decomposition is shown in Fig. 5.

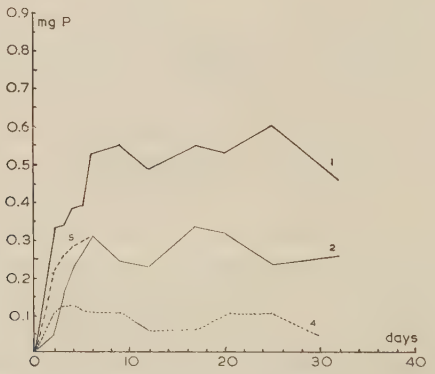

Fig. 5. Sample 2457. Graphs 1 to 5 as under Fig. 3. Zero values for Graph 4.

There was a marked build up of microbial organic phosphorus during the first six days after which approximately equal amounts of acid-labile phosphorus (Graph 2) and acid-stable phosphorus (Graph-1 values minus Graph-2 values) were present. All the organic phosphorus present at the various stages of decomposition was hydrolysed during 5 days with hot *N* sulphuric acid. No cold-

acid-extractable phosphorus in excess of water-soluble inorganic phosphorus was obtained throughout the decomposition period.

The absence of any marked fluctuations in the various fractions, such as was found with samples 756 and 33, after the sixth day, indicates a steady state in the microbial population. It appears that after the initial build-up of bacterial organic phosphorus any inorganic phosphorus subsequently produced through dephosphorylation is utilised by newly developing bacteria, this process continuing as long as there is available substrate to the decomposed. The persistance of slowly decomposable substrate with mature plant material was mentioned earlier.

When the samples were treated with chloroform prior to extraction with water a small proportion of the bacterial organic phosphorus was dephosphorylated to inorganic phosphorus (Graph 3), which remained fairly constant throughout the decomposition process. This indicates the presence of viable cells at a relatively low and constant level and supports the hypothesis outlined in the previous paragraph namely, that as old cells die off, the inorganic phosphorus released is utilised by newly developing bacteria which in turn die off and release inorganic phosphorus, this process continuing as long as substrate is available for decomposition. It is probable that when the substrate is eventually exhausted dephosphorylation of bacterial organic phosphorus according to the processes found for Samples 33 and 756 will occur.

When the samples were exposed to chloroform vapour and then extracted with hot acid the amount of acid-labile phosphorus obtained (Graph 4) during the first four days was greater than from the non-chloroform-treated samples after which there was no difference between the two treatments. If the effect of chloroform was to bring about enzymatic hydrolysis of acid-stable phosphorus Graph 4 would be above Graph 2 after the fourth day. Hence it appears that only prior to the fourth day are acid-stable forms enzymatically hydrolysed on death of the cells to acid-labile forms.

Experiment 5. Inorganic phosphorus fluctuations during glucose decomposition

Solutions were prepared such that 6.0 ml contained the amounts of carbon (as glucose), nitrogen (as ammonium sulphate), and phosphorus (as potassium dihydrogen phosphate) shown in Table 4. Each solution contained, in addition, 2.4 mg of magnesium sulphate.

TABLE 4

Solutions used for glucose decomposition experiments							
Treatment No.	mg C	mg N	mg P	C : N	C : P	N : P	glucose decomposition (32 days), %
1	96	1.2	1.080	80	89	1.1	97.0
2	96	9.6	0.216	10	444	44.5	81.6
3	96	9.6	0.864	10	111	11.0	98.8

Each of the solutions was added to 50 g sand plus 0.1 g soil (as inoculum) and incubated at 25°C in the respirometer. Two-gram samples of each treatment were subsequently withdrawn at intervals for the determination of water-soluble inorganic phosphorus. The effect of exposure to chloroform vapour (prior to extraction) on the amount of inorganic phosphorus extracted and the amount of glucose decomposed at various stages, were also followed.

Fungi were found to be largely involved in the decomposition processes and, in contrast to bacteria, gave little extra inorganic phosphorus after chloroform treatment. Work here indicates that fungi are not susceptible to this treatment.

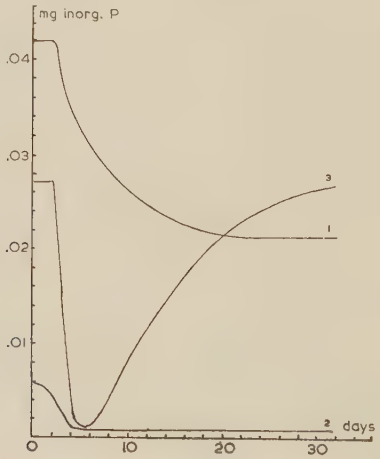

Fig. 6. Inorganic-P fluctuations during glucose decomposition. P as mg P per 2-g sample removed at intervals. Curves 1 to 3 refer to treatments in Table 4.

Fig. 6 shows no recovery of inorganic phosphorus with Treatments 1 and 2 where, because of low amounts of N and P respectively early decomposition is slower than with Treatment 3 and more

prolonged. This result is parallelled by Group-2 plant samples where decomposition is also prolonged, but in this instance because of the poor availability of the substrate.

Treatment 3, in which initial decomposition was rapid resulted in an almost complete recovery of inorganic phosphorus. Here it appears, as with the Group-1 plant samples, that a large microbial population is initially built up and, having utilised most of the substrate, dies off with release of inorganic phosphorus as lysis occurs. The high proportion of glucose decomposed even under low inorganic-phosphorus conditions is also noteworthy (Table 4).

DISCUSSION

The foregoing work demonstrates that recovery of microbial organic phosphorus, synthesised from plant inorganic phosphorus during the early stages of decomposition, depends largely on rapid decomposition and exhaustion of substrate leaving a large population to undergo autolysis with the release of inorganic phosphorus through enzymatic dephosphorylation processes. Substrate causing such behaviour is afforded by young plant material. With mature material the microbial population built up initially is smaller and the more prolonged supply of less readily available substrate maintains a fairly uniform cycle of microbial growth and decay in which any microbial organic phosphorus mineralised is used again. With both young and mature material however no pronounced mineralisation of plant organic phosphorus appears to take place during decomposition periods up to three months.

Chang [9] states that the formation and mineralisation of organic phosphorus complexes seem to depend on the amount of available energy-supplying materials, which are themselves influenced more by the age than the nature of the plant. He also remarks that when the materials which supply available energy become exhausted the micro-organisms attack complex organic phosphorus compounds including those originally present in the plant. There is however no evidence with the above samples that this stage is reached even after three months, when decomposition is virtually complete. It is possible that mineralisation of plant organic phosphorus is masked by microbial uptake of the inorganic phosphorus produced. Maximum mineralisation of plant organic phosphorus would,

however, be expected to occur during the early stages of decomposition when microbial activity is high, yet when the bacterial cells were killed during this period no more inorganic phosphorus was recovered than was present before decomposition, indicating that little or no mineralisation of organic phosphorus had occurred. From the plant decomposition studies and Experiment 5 (which shows similar patterns of behaviour although no organic phosphorus was originally present) it appears that phosphorus transformations involving interactions between bacteria and the inorganic phosphorus initially present are of greater significance than the mineralisation of plant organic phosphorus which appears to occur only slowly.

Kaila [10] using natural organic materials found an increase in organic phosphorus over a three-month decomposition period with rye straw and wheat roots, and little change with horse dung. Mineralisation of organic phosphorus initially present in the material occurred however with wheat bran, fungal material, cattle manure and green fodder, which contained 0.78, 0.27, 0.22, and 0.15 per cent organic phosphorus respectively. The only plant material containing a comparable amount of organic phosphorus in the present work was Sample 745 (0.21 per cent) which, from Table 3, may have undergone slight mineralisation after three months decomposition. In general with plant material we are dealing with organic-phosphorus contents lower than this, but decomposition is not hindered since the supply of inorganic phosphorus is sufficient and can, if necessary, be re-utilised during decomposition as it becomes available from dead micro-organisms. In this connection it may be noted that Caldwell and Hinshelwood [8] found that the inorganic phosphate in the medium had to become almost completely exhausted before any serious effect on the growth rate of *Bact. lactis aerogenes* could be detected. (*cf* Col. 8, Table 4).

Changes in the various phosphate fractions constituting the bacterial organic phosphorus are described in Experiment 4, in which two stages are apparent in the dephosphorylation of microbial organic phosphorus namely (a) hydrolysis of acid-labile phosphorus followed by (b) that of acid-stable phosphorus. According to Bartholomew and Goring [2] phosphorus release and cell decomposition (of the cell suspension used in their experiments) was in

part the result of autolysis and in part the result of decomposition by other micro-organisms. Possibly stage (a) above is related to autolysis and stage (b) to decomposition by a freshly developing population using the lysed products. This is to some extent supported, with Sample 33, by the reappearance of acid-labile and acid-stable forms of phosphorus, as well as by a response to chloroform vapour, such as would be expected with a newly developing population. Alternatively stage (a) may be due to internal changes in ageing cells and stage (b) to autolysis itself.

Bartholomew and Goring [2] found that decomposition of the cells resulted in some breakdown of all the organic phosphorus complexes. A similar result was found here and in one instance (Expt. 4, Sample 33) almost complete dephosphorylation of both acid-stable and acid-labile compounds occurred. After about five weeks decomposition with sample 33 and 756 only 21 and 31 per cent respectively of the maximum amount of microbial organic phosphorus synthesised earlier remained, consisting of approximately 70 per cent acid-labile phosphorus and 30 per cent acid-stable phosphorus. The declining portions of Graph 1 (Figs. 3 and 4) are of an asymptotic nature and it appears that with time and under uniform moist conditions both these forms would eventually be mineralised. With Sample 2457 after 5 weeks decomposition about 81 per cent of the maximum amount of microbial organic phosphorus formed was still present, made up of approximately equal amounts of acid-labile and acid-stable phosphorus. These conditions are likely to persist as long as substrate is available but eventually transformations of the kind found in the decomposition of Samples 33 and 756 are likely to occur. Hence the plant inorganic phosphorus tied up as microbial organic phosphorus during decomposition may all be recovered as inorganic phosphorus (provided that death and lysis of the micro-organisms follows an uninterrupted and normal course) however difficulty hydrolysable by chemical methods these compounds may be. These experiments were however carried out in a sand medium whereas in soil protection from decomposition through adsorption of the organic phosphorus compounds or of the enzymes may afford some protection of these potentially mineralisable compounds.

The high proportion of phytin found by Bower [5] in the organic-phosphorus fraction of soils is of plant origin (since it is not a

constituent of micro-organisms) and further supports the observations made above that plant organic phosphorus compounds are little mineralised during plant decomposition presumably because of their resistance to decomposition. The overall result of plant decomposition appears to be, from the phosphorus aspect, the formation of residual compounds consisting of largely unchanged plant organic phosphorus together with acid-stable and acid-labile microbial organic phosphorus in amounts and proportions depending on the material decomposed (young or mature), the stage at which decomposition stopped and, under natural conditions, the influence of the soil.

There is little in the literature on the chloroform effect described above, but Stickland [13] found that the rate of liberation of inorganic phosphorus after poisoning of the esterifying systems of yeast with urethane was of a similar order of magnitude to the rates of esterification. A similar result was obtained here during the early stages of decomposition when exposure of the cells to chloroform vapour for 24 hours was sufficient to convert to inorganic phosphorus most of the organic phosphorus formed during the previous day (Figs. 3 and 4).

The conversion of inorganic to organic phosphorus during substrate utilisation, followed by dephosphorylation, is of wide occurrence. According to Brodie and Gray [6] the coupled oxidative phosphorylation observed with the bacterial system is similar to that in mammalian tissue, and utilisation of inorganic phosphorus probably involves the same type of mechanism. Macfarlane [11] found with yeast that when the free sugar had disappeared there was a reappearance of inorganic phosphorus in the cell. Mann [12], dealing with the phosphorus metabolism of moulds, found that mould fungi adsorb ortho phosphate aerobically and convert it to a number of organic compounds a large proportion of which is converted to acid-hydrolysable compounds. During autolysis of mycelium organic phosphorus compounds were decomposed and excreted into the medium as ortho phosphate. A similar result was obtained in Expt. 5, with Mixture 3, in the decomposition of which fungi were largely operative.

SUMMARY

During plant decomposition microbial organic phosphorus is rapidly formed from plant inorganic phosphorus. Subsequently with young plant material much of the microbial organic phosphorus is dephosphorylated and recovered as inorganic phosphorus. With mature material there was little recovery even after three months decomposition. Recovery depends on rapid utilisation and exhaustion of easily decomposable substrate leaving a relatively large microbial population to die and undergo lysis. Poor recovery is associated with less-decomposable substrate and the maintenance of a viable population.

In no instance was more inorganic phosphorus recovered after up to three months decomposition than before. Plants generally contain sufficient inorganic phosphorus for bacterial requirements during decomposition so that supplementary supplies obtained by mineralisation of plant organic phosphorus are unnecessary.

During dephosphorylation of microbial organic phosphorus acid-labile forms are first involved and then the acid-stable forms. Secondary decomposition possibly involving dead micro-organisms results in the resynthesis of both forms (though to a much smaller extent than during the early stages of decomposition). These however suffer further dephosphorylation and the residual products consist roughly of 70 per cent acid-labile and 30 per cent acid-stable phosphorus. With mature material the amount of organic phosphorus remains fairly constant during five weeks decomposition and consists of approximately equal parts of both forms.

Exposure of the samples at progressive stages of decomposition to chloroform vapour resulted in rapid dephosphorylation of the microbial organic phosphorus during the early stages of decomposition when microbial enzyme activity is high. Enzyme systems converting acidstable and acid-labile forms to inorganic phosphorus, and acidstable to acid-labile forms could be distinguished.

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SOIL AGGREGATION AND LIME CONTENT AS FACTORS IN LIME-INDUCED CHLOROSIS

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Soil aeration determines to a large extent the intensity of chlorosis in plants growing on highly calcareous soils [13]. Poor soil aeration results in decreased partial pressure of oxygen and increased pressure of carbon dioxide, thus increasing the concentration of the bicarbonate ion in the soil solution. In studies with plants grown in nutrient solutions [2] an increased concentration of the bicarbonate ion resulted in chlorotic plant symptoms, resembling those found on highly calcareous soils. Brown, in his review [1], assumes that this ion plays a similar role in calcareous soils. Therefore, it seems likely that poor soil aeration should aggravate chlorotic symptoms in plants.

On the other hand, there is evidence from nutrient solution studies [3 5] that a positive relation consists between chlorosis and oxygen content in the bubbling gas. The present paper represents an attempt to clarify the conflicting evidence and to evaluate the role of soil aggregation in the formation of chlorotic plant symptoms. Sensitive and resistant plants were investigated on soils of different lime content.

MATERIALS AND METHODS

Plants

Dallis grass (*Paspalum dilatatum*) was used as the sensitive, and Rhodes grass (*Chloris gayana*) as the resistant indicator plant. To improve uniformity, sod of both species was taken from adjacent plots of an irrigated pasture. Single plants were extracted from the blocks of sod, and their leaves cut back. The plants were immersed in tap water for 24 hours and planted in 2-kg soil containers.

Soil

Four different levels of lime content were produced by mixing a grey mountain rendzina (Wadi Ara) and an alluvial soil (Zarnuga). Both soils were crushed to pass a 1-mm sieve and mixed in the required proportions. To prepare stable aggregates, a synthetic soil conditioner (HPAN, 0.2 per cent) was added to the soil mixtures. The soil was moistened, worked over by hand, and then air dried. Part of each mixture was crushed to pass a 1-mm sieve, and part screened through a 15-mm sieve, resulting in fine and coarse aggregates respectively.

The four soil mixtures differ mainly in total and colloidal lime content as well as in the mean weight diameter of their particles. Their other properties are similar; although there are significant differences in moisture equivalent between fine and coarse aggregates, they are very slight.

Two kg soil were filled into earthen pots covered internally and externally with plastic paint. 2.5 g superphosphate, 0.5 g potassium chloride and 1 g ammonium sulphate were mixed with the soil of each container prior to filling. A further 1 g of ammonium sulphate was added after the first month of growth.

TABLE 1

Properties of the soil mixtures used in the experiment

Calca-* reous soil in the mixture %	Aggregate size	Moisture equivalent %	Pw air dry %	Total** lime %	Active*** lime %	pH⁰	Specific†† surface m^2/gm	Cation † exchange capacity meq./100 gm	Mean † weight diameter
0	fine	27.5	4.5	2.6	2.0	7.70	165	21.5	0.405
	coarse	26.0			2.0	7.60	177		2.390
33	fine	27.5	4.4	26.3	11.5	7.65	173	21.0	0.368
	coarse	25.8			12.0	7.60	181		2.410
66	fine	28.1	4.3	47.2	21.5	7.60	182	20.7	0.410
	coarse	26.0			22.5	7.70	169		2.000
100	fine	27.6	4.5	73.3	32.5	7.65	180	20.0	0.430
	coarse	25.8			31.5	7.65	175		1.350

* Made up to 100 percent by alluvial soil

** Gasometric determination

*** Determined as described in Ref [4]

† Determined as described in Ref.[11]

†† Determined as described in Ref.[10]

Oxygen and carbon dioxide analysis.

Glass tubes with an inner diameter of 12 mm were inserted into each soil container to a depth of 10 cm. To their upper ends 3-ml sampling ampoules were connected with rubber tubing and left for at least 24-hours to reach equilibrium. Then the rubber tube was clamped tight and, together with the ampoules, disconnected from the soil tube. Air at the mouth of the rubber tubing was displaced with a drop of mercury. The oxygen and carbon dioxide in the ampoules were analyzed by the Van Slyke method [14].

All soil containers were examined at four dates, and significant differences were found only between the two aggregate sizes. No differences could be found between the four mixtures of the same aggregate size. Average results of the two structural groups were given (in volume percents) in Table 2.

TABLE 2

Structure	O_2 % – volume	CO_2 % – volume
Coarse	19.5 ± 1.3	0.95 ± 0.37
Fine	16.9 ± 0.8	2.00 ± 0.30

The diffusion of oxygen in the soil was measured according to Taylor [12]. At field capacity, 2.9 and 3.9 cm were found to be the effective diffusion distances for the coarse and fine fraction respectively.

Plant analysis.

The plants were harvested after a growth period of twelve weeks. The roots were washed thoroughly in running tap water and separated from the leaves. Both leaves and roots were oven dried for 48 hours at 80°C and their separate fresh and dry weights determined. Chlorophyll was extracted by 80% acetone and measured by Mackinney's method [6]. Results were expressed as a percentage fresh weight, dry weight and leaf surface.

Leaf and root respiration were determined with a Warburg apparatus at 25°C. For the measurement, roots were immersed in 2.5 ml Arnon-Hoagland solution diluted to one third; respiration of the leaves was determined in the dry flasks.

RESULTS

Only the Dallis grass showed a significant response to the experimental treatments; no response was shown by Rhodes grass. The dry weight percentages of both leaves and roots were significantly lower in the high lime soil mixtures of the coarse aggregates (Table 3); the decrease in the fine aggregates is slight and not significant.

TABLE 3

Effect of the soil mixtures on the dry weight percentage and weight per unit area of Rhodes and Dallis grass.

Calcareous soil in the mixture %	Dallis grass				Rhodes grass		
	Leaf mg F.W.* per cm²	Leaf mg D.W.** per cm²	Leaves D.W.** %	Roots, D.W.** %	Leaf mg F.W.* per cm² %	Leaf mg D.W.** per cm²	Leave D.W.* %
Fine aggregates							
0	16.26	3.80	23.2	22.0	16.9	4.25	22.0
33	15.34	3.39	21.5	25.6	18.1	4.15	22.1
66	16.00	3.59	22.2	20.0	17.2	3.95	22.0
100	15.89	3.38	21.0	18.2	17.5	4.60	20.3
L.S.D. 0.05	2.41	0.86	5.2	7.1	3.2	0.85	1.9
Coarse aggregates							
0	13.82	3.30	24.0	30.8	17.4	4.45	23.1
33	15.46	3.20	21.3	28.7	17.7	4.30	20.8
66	15.20	2.75	17.6	23.1	17.4	4.10	21.0
100	15.41	3.50	22.0	21.4	16.9	4.23	19.3
L.S.D. 0.05	1.97	1.87	2.87	5.2	2.8	0.72	4.6
L.S.D. 0.05 *total*	2.03	0.97	4.07	6.8	2.9	0.80	4.2

* F.W. = Fresh weight
** D.W. = Dry weight.

The weight per unit area of the leaves was uniform in all treatments. Regarding chlorophyll, its content in Dallis grass leaves was lowered by increasing lime content in the coarse soil only (Table 4).

Analysis of variance of the chlorophyll data (Table 5) shows the linear regression of chlorophyll content as a function of lime content in the coarse mixtures to be significant at 0.01 level (except for chlorophyll-b on a percentage of dry weight basis and the chlorophyll a/b ratio). The table indicates also the significant interaction between lime and aggreagate size upon their effect on chlorosis. The differences between coarse and fine aggreagates were significant at $P = 0.01$.

The oxygen uptake of Dallis grass roots was depressed in the high-lime treatments in coarse soil only (Fig. 1), as shown by respiration measurements. CO_2 release was affected likewise. In all other treatments there was no effect on respiration.

The results for Dallis grass on coarse aggregates were given a

TABLE 4

Effect of the soil mixtures on chlorophyll content in Dallis grass leaves

Calcareous soil in the mixture %	Chlorophyll mg/g F.W.*			Chlorophyll mg/g D.W.*			Chlorophyll mg/100 cm²			Chloro-phyll *a*/*b* ratio
	a	*b*	*total*	*a*	*b*	*total*	*a*	*b*	*total*	
Fine aggregates										
0	1.59	0.52	2.11	6.34	2.24	8.58	2.25	0.87	2.13	2.83
33	1.45	6.69	2.14	7.14	3.42	9.13	2.28	1.09	3.38	2.17
66	1.62	0.70	2.32	7.56	3.26	10.82	2.55	1.09	3.64	2.37
100	1.65	0.62	2.25	7.93	3.56	11.48	2.51	1.13	3.64	2.65
L.S.D. 0.05	0.38	0.24	0.72	3.50	2.02	4.38	0.99	0.40	1.07	0.17
Coarse aggregates										
0	1.40	0.64	2.06	6.67	2.32	9.00	2.20	0.94	3.15	2.51
33	0.97	0.39	1.36	4.87	1.96	6.83	1.54	0.62	2.15	2.48
66	0.87	0.25	1.39	4.89	2.09	6.97	1.23	0.51	1.74	2.51
100	0.63	0.28	0.91	2.89	1.12	3.99	1.04	0.46	1.50	2.26
L.S.D. 0.05	0.36	0.21	0.79	1.77	2.40	2.43	0.60	0.29	0.88	0.45
L.S.D. 0.05 *total*	0.37	0.22	0.47	2.40	1.64	3.43	0.88	0.40	1.10	0.20

* See footnotes to Table 3

TABLE 5

Analysis of variance of the chlorophyll data presented in Table 3.

Dependent variable	Source of variation			
	Linear regression in coarse aggregates	Interaction lime-aggregate size	Difference, fine and coarse aggregates	Error mean square
Chlorophyll *a* mg/g F.W.†	1.597**	1.106**	3.150**	0.100
,, *b* ,, ,,	0.428**	0.357**	0.798**	0.0285
,, *total* ,, ,,	3.979**	4.836**	6.573**	0.338
Chlorophyll *a* mg/g D.W.†	32.01 **	36.25 **	7.74	4.55
,, *b* ,, ,,	2.67	7.41 *	3.27	1.65
,, *total* ,, ,,	63.74 **	95.86 **	135.02 **	8.61
Chlorophyll *a* mg/100 cm²	79.54 **	72.20 **	82.41 **	3.79
,, *b* ,,	4.91 **	6.54 **	20.96 **	0.78
,, *total* ,,	68.51 **	58.60 **	206.50 **	5.82
Chlorophyll *a*/*b* ratio	0.136	0.021	0.187	0.249

* Significant at $P = 0.05$

** ,, at $P = 0.01$

† See footnotes to Table 3.

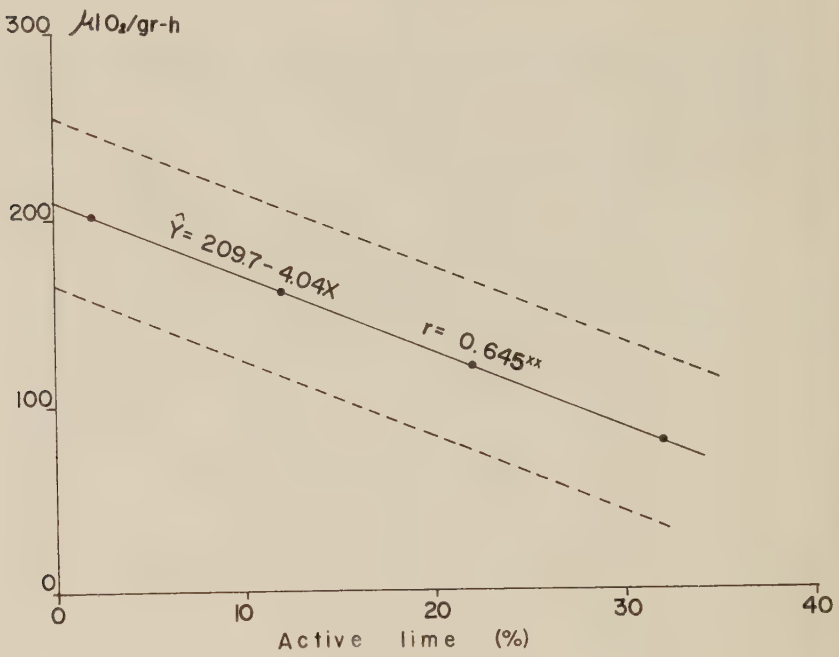

Fig. 1. Oxygen uptake of Dallis grass roots in the coarse soil mixtures μl O_2/g/hour (fresh-weight basis).

TABLE 6

Correlation between dry weight percentage, weight per surface area unit and chlorophyll content of Dallis grass leaves and their root oxygen consumption in coarse soil.

Dependent variable	Regression	Correlation coefficient (r)
Dry weight, %	—	0.202
Leaves, mg/100 cm² D.W.†	—	0.405
Leaves, ,, F.W.†	—	0.256
Chlorophyll *a* mg/g F.W.†	$\hat{Y} = 0.748 + 0.000035\ X$	0.625**
,, *b* ,, ,,	$\hat{Y} = 0.256 + 0.00021\ X$	0.652**
,, *total* ,, ,,	$\hat{Y} = 0.948 + 0.00080\ X$	0.721**
Chlorophyll *a* mg/g D.W.†	$\hat{Y} = 0.484 + 0.00202\ X$	0.755**
,, *b* ,, ,,	$\hat{Y} = 1.441 + 0.00073\ X$	0.700**
,, *total* ,, ,,	$\hat{Y} = 4.800 + 0.00302\ X$	0.791**
Chlorophyll *a* mg/100 cm²	$\hat{Y} = 1.190 + 0.00050\ X$	0.616**
,, *b* ,,	$\hat{Y} = 0.474 + 0.00024\ X$	0.638**
,, *total* ,,	$\hat{Y} = 1.588 + 0.00088\ X$	0.750**
Chlorophyll *a*/*b* ratio	—	0.408

† See footnotes to Table 3.

further examination. Though no significant correlation was found between either fresh or dry weight or weight per unit area and the oxygen uptake of roots (Table 6), the correlation between chlorophyll content of the leaves and oxygen consumption was found to be highly significant. The correlations were highest with chlorophyll content expressed on a dry weight percentage basis which, in this case, seems to be the most stable and sensitive response indicator.

DISCUSSION

There are two variables in the experiment – aggregate size and lime content of the soil. In the following discussion they will be dealt with separately at first.

A. *Aggregate size or soil structure.*

As the experimental results show, aggregate size influences the properties of all three soil phases, namely the mean-weight diameter of particles, O_2 and CO_2 contents the oxygen diffusion rate, and to a lesser degree the moisture equivalent. At the effective diffusion distance of 3.9 cm found in the finely aggregated soil, plants may suffer from lack of oxygen [12]. In this connection it is important to note that, according to the experimental results, the four soil mixtures of the same aggregate size were identical in their structural properties.

B. *Lime content.*

Lime content is easy to define as a variable, but which of its many aspects affects plant growth is extremely difficult to determine. As shown in Table 1, rising increments of Wadi Ara soil raise the percentage of total and active lime in the soil mixture but do not affect its pH, cation-exchange capacity and specific soil surface. There is no assurance, however, that other soil parameters, not measured, remained unchanged. Even assuming that all the various indices relating to the lime factor were measured, we will still find that we have not determined whether the chlorosis is caused by the $CaCO_3$ salt, the Ca^{++} or CO_3^{--} ions, or by the HCO_3^- and OH^- ions, resulting from hydrolysis of the $CaCO_3$. It may be that the chlorosis is a resultant of a combination of two or more of the above factors.

The experimental results indicate that lime content had an effect on plant growth only in the case of Dallis grass growing in coarsely aggregated soil. The similar trend of Dallis grass roots and leaves in the three low-lime treatments is clear evidence of the effect of lime content on the dry weight of the plant. The different dry weights of roots in the coarse and fine fraction seem to indicate the importance of aeration, or a similar factor in root dry-matter production. Root respiration measurements showed that their O_2 uptake, and possibly CO_2 release, were reduced by high lime content. This reduction agrees with the respiration inhibition caused directly [7] or indirectly [8] by the HCO_3^- ion. This inhibition may give rise to further changes in the plant, resulting finally in a lowering of the chlorophyll level in the leaves. The linear correlation found between chlorophyll content and root respiration strengthens this assumption.

The reaction of Dallis grass to an increased lime content in the soil as measured by its leaf chlorophyll level was different on the coarse and fine soil mixtures. Leaf chlorophyll content was lower on the coarse than on the fine soil at the same lime content, except in the low-lime treatment. While in the coarse fraction leaf chlorophyll was reduced by a higher lime percentage in the soil mixture no such effect was found on the fine aggregates. Therefore, a soil structure—lime content interaction regarding chlorosis may be assumed. This assumption seems to be substantiated by the results presented in this paper (Table 5), both for chlorophyll *a* and *b*, as expressed on a dry weight, fresh weight and leaf area basis. The soil factor influences chlorophyll *a* and *b* identically and there is no significant change in their ratio.

In the concluding part of the discussion an attempt will be made to explain the interaction found experimentally on the basis of the soil parameters examined, and differing on the coarse and fine soil mixtures. Differences were recorded in the mean-weight diameter of their particles, O_2 and CO_2 percentage in the soil atmosphere, and the diffusion of oxygen.

At low soil-moisture tensions poor aeration reduces the growth of sunflowers in very fine soils, as stated by Miller and Mazurak [9], while in coarse soils a poor root—soil solution contact is the limiting factor. At higher tensions, that is tensions at field capacity, the soil water content no more impedes oxygen diffussion

significantly, and poor contact seems to be the reason for reduced growth in both fine and coarse soils, growth being best in the finest, and lowest in the coarsest fraction. As the soils in this experiment were kept at a moisture content close to field capacity by daily irrigations and the mean particle diameter of the two aggregate sizes corresponds to the coarse and medium fractions in Miller and Mazurak's experiment, it seems justified to ascribe the lowered dry weight, surface weight irato, and chlorophyll content of Dallis grass grown on the coarse soil to a poor root—soil solution contact. The HCO_3^- ion, a product of the hydrolytic dissociation of lime, is generally assumed to be reponsible, even if indirectly, for chlorosis in plants. Lime content, the partial pressure of CO_2 and the pH determine the bicarbonate concentration in the soil solution. Since lime content does not change, and the $CaCO_3—H_2O$ system being a strong buffer, the pH is liable to be stable, the bicarbonate ion concentration is governed by CO_2 partial pressure only. In fine-textured soil gaseous diffusion is slow, resulting in a raised CO_2 level; also, root contact with the soil solution and its bicarbonate ion is more intimate than in coarse soils and the solution of CO_2 released by the roots is facilitated. The soil moisture content at equal tensions is somewhat higher in fine than in coarse soils, thus increasing the lime solubility, reducing the gaseous diffusion rate, and increasing root contact. We may expect, therefore, chlorosis to be more pronounced on fine textured soil. As the experimental results do not agree with this deduction, a structural factor other than CO_2 partial pressure would appear to be active in the lime—soil structure interaction causing chlorosis. This factor is liable to be the oxygen concentration in the soil atmosphere, or its diffusion rate, or any secondary cause influenced by them. This point requires further clarification.

ABSTRACT

Lime-induced chlorosis of Dallis grass was caused by an interaction of structure and lime content of the soil. Root respiration was affected by lime content on coarsely aggregated soil only. A correlation between root respiationr and leaf chlorophyll content was found, but no causal relationship is necessarily to be inferred. The nature of the lime-soil structure interaction, possibly an aeration factor, requires further clarification.

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THE EFFECT OF PARTIAL PRESSURE OF OXYGEN IN FIELD SOILS ON LIME-INDUCED CHLOROSIS

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INTRODUCTION

Baver mentions in his book [1] that restricted soil aeration is the greatest limiting factor in the development of an excessive root system, and that it prevents the orderly functioning of essential biological processes associated with good soil fertility. It has previously [3 5 7] been proven that, at least where the danger of chlorosis is involved, Baver's statement is not entirely correct.

Proof of the damaging effect of high partial pressure of oxygen was obtained from solution culture and pot experiments [3 5 7]. In order to relate such experimental results to the behaviour of plants growing in the field, it is essential to verify the conclusions reached under field conditions.

While the control of the partial pressures of various gases is possible in containers, this is not possible under field conditions. For this reason it was decided to examine the soil atmosphere of plots which were identical from the macroecological point of view, with no soil differences due to lime content, texture, pH, *etc.*, whose vegetative cover nevertheless showed various degrees of chlorosis.

MATERIALS AND METHODS

Plants

The plants chosen as indicators of the effect of soil conditions on chlorosis were Dallis grass (*Paspalum dilatatum*) in an irrigated pasture at Yokneam (western Jezreel Valley).

Soils

The properties of the soils are summed up in Table 1. The data of the table show that there are almost no differences among the plots on the basis of the properties tested, and that therefore various degrees of chlorosis could not have been caused by differences in these properties.

TABLE 1

Plant condition	Horizon cm	Properties of the soil: Hygroscopic moisture	Total* lime %	Active** lime %	pH***	E.C.†of*** extract mmhos/cm	Clay†† (< 2μ) %	Organic** matter
Chlorotic plot A	0 — 30	10.0	7.2	6.7	7.7	1.5	48	2.4
	30 — 60	10.5	7.4	6.8	7.7	1.7	52	1.4
Chlorotic plot B	0 — 30	10.6	6.8	6.9	7.7	1.6	49	2.3
	30 — 60	10.5	7.2	6.8	7.8	1.8-	50	1.5
Green plot A	0 — 30	9.8	7.4	7.5	7.6	2.0	45	2.5
	30 — 60	10.5	7.5	7.7	7.7	2.4	49	1.4
Green plot B	0 — 30	10.0	7.8	7.7	7.7	1.8	50	2.3
	30 — 60	10.4	8.1	7.9	7.8	2.1	52	1.6

* Gasometric method
** Drouineau [4].
*** Richards [8]
† Electrical conductivity.
†† Sudan method.

Experimental plots

The field in Yokneam is an irrigated pasture sown to Dallis and Rhodes grass. To the east of the plot there is a drainage ditch about two metres deep, which is dry during the summer months, and today is moreover not in use during the other seasons as well. On the southern side of the plot there is a shallow depression, which was the boundary of a swamp before the area was brought under cultivation. The area was levelled before sowing.

The plot was irrigated by a portable sprinkler system using rotating sprinklers mounted on laterals running east—west. Soil moisture determinations and visual observation indicated uniform distribution of water along the lateral, except for the vicinity of the part-circle sprinkler mounted on the western end of the lateral. Irrigations were applied every 10 to 12 days.

Along the eastern drainage ditch there is a strip of very chlorotic plants. The colour of the vegetation becomes greener with increasing distance from the ditch, and at a distance of some five metres the plants are green and healthy. As one approaches the depression described above, chlorotic plants are again in evidence. Another chlorotic area about four to five metres wide

is found on the other side of the depression, while in the depression itself the plants are green and altogether healthy.

Four test plots were chosen along the line of the irrigation lateral. One plot, *"chlorotic A"*, was located in the chlorotic strip along the eastern ditch. Plot *"chlorotic B"* was located on both sides of the depression, whereas the depression itself was named *"green A"*, and another green area at the western end of the irrigation lateral was called *"green B"*. In each area there were installed at random locations six diffusion wells at three depths: 20 cm, 30 cm, and 60 cm. Installation of the wells and sampling technique were carried out according to van Bavel [10]. Oxygen analyses were carried out for one month, which comprised three irrigation cycles.

Leaf analysis was carried out on the Dallis grass leaves. In each sampling area three clumps of plants were chosen, the leaves were cut, chopped, and thoroughly mixed. From this material two sub-samples of two grams each were removed, and the chlorophyll was extracted from them while still fresh in the field, using 80% acetone [6]. The test tubes of extract were transported to the laboratory in thermos containers full of ice. In the laboratory the samples were warmed to room temperature, and their optical density was determined with a Beckman Model DU spectrophotometer according to the Mackinney method [6].

Since chlorotic plots were selected according to the colour of the plants – the chlorophyll content of the leaves was considered as the best quantitative measure of chlorosis.

Leaf area and dry matter determinations were carried out as described previously [7].

RESULTS

Differences in chlorophyll in the Dallis grass leaves from the four sampling areas are shown in Table 2.

TABLE 2

Chlorophyll content (mg) of Dallis grass leaves

Chlorophyll type, and basis of computation	Plot			
	Chlorotic A	Chlorotic B	Green A	Green B
Chl. *a* per g fresh weight	0.38 ± 0.19	0.35 ± 0.17	1.65 ± 0.42	2.02 ± 0.57
,, *b* ,, ,, ,,	0.20 ± 0.05	0.21 ± 0.09	0.78 ± 0.18	1.09 ± 0.31
,, *total* ,, ,, ,,	0.57 ± 0.20	0.57 ± 0.18	2.47 ± 0.51	3.09 ± 0.62
,, *a* per g dry weight	2.07 ± 0.42	1.69 ± 0.39	6.62 ± 1.61	6.91 ± 1.52
,, *b* ,, ,, ,,	0.98 ± 0.21	0.90 ± 0.28	3.01 ± 0.57	2.90 ± 0.73
,, *total* ,, ,, ,,	3.10 ± 0.67	2.62 ± 0.59	9.73 ± 1.91	9.02 ± 2.00
,, *a* per 100 cm^2	0.55 ± 0.23	0.51 ± 0.24	2.41 ± 0.60	2.61 ± 0.61
,, *b* ,, ,, ,,	0.27 ± 0.09	0.30 ± 0.13	1.15 ± 0.27	1.08 ± 0.30
,, *total* ,, ,, ,,	0.85 ± 0.31	0.82 ± 0.29	3.61 ± 0.79	3.80 ± 0.71

Table 2 shows that there are no significant differences between the chlorophyll content of the plants that grew along the eastern ditch (see Methods) and those that grew on the edges of the depression. On the other hand, comparing the chlorophyll content of the green plots on a fresh-weight basis it appears that the plants from the western part of the field were greener than those from the depression. This impression is invalidated by inspection of the data computed on a dry-weight basis. The different results obtained for the different bases are explained by differences in hydration of the leaves (Table 3).

TABLE 3

Percent dry matter and weight per unit area of Dallis grass leaves				
Parameter	Plot			
	Chlorotic A	Chlorotic B	Green B	Green B
Dry matter, %	20.5 ± 2.0	21.6 ± 2.1	25.4 ± 2.7	29.7 ± 3.5
Weight/area (fresh) *	14.6 ± 2.1	14.3 ± 1.7	13.8 ± 1.9	12.2 ± 1.6
Weight/area (dry)*	3.3 ± 0.7	3.4 ± 0.4	3.7 ± 0.9	3.8 ± 1.1

* mg/cm^2

The differences in dry-matter content between areas "green A" and "green B" indicates that the apparently higher chlorophyll content in the leaves from the latter area is due to the lower moisture content. The same can also be determined by a comparison of the data referring to weight per unit area computed on a dry- and fresh- weight basis. Table 3 also confirms the absence of significant differences between the two chlorotic areas.

It is interesting to note the opposite trends of the weight per unit area as computed on a fresh- or dry-weight basis (on a fresh-weight basis it increases with chlorosis, and on a dry-weight basis it decreases).

In Figures 1, 2, and 3, the changes in the partial pressure of oxygen are presented as an average of the two values obtained from the two chlorotic plots, whereas for the green plots only data from plot "green A" are presented, since it does not seem justified to compare plots with a different water regime.

Fig. 1 is interesting in particular because of the shallow rooting habit of Dallis grass. The majority of the roots ($\sim 50\%$) are found within the 0- to 20-cm depth. A comparison of the green and chlo-

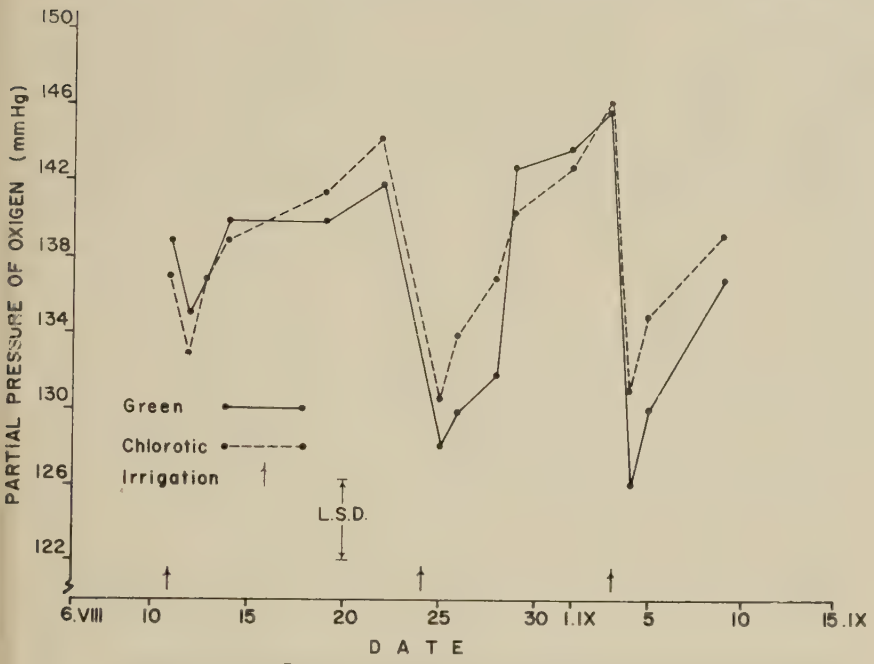

Fig. 1. Partial pressure of oxygen in the soil atmosphere at a depth of 20 cm.

rotic plots shows that the difference between them is very small. Significant differences in oxygen concentration between the two plots are apparent on two occasions, (26 to 28/8, and 4 to 7/9), both times immediately following irrigation, when the soil moisture was still very high and the oxygen concentration relatively low. At a greater depth the difference between the two plots is greater, as may be seen from the following figure.

At the 30-cm depth the level of oxygen is higher in the chlorotic areas than in the green ones throughout the sampling period. While the difference is not significant for all sampling dates, it is so much more frequently than for the 20-cm depth. Between the 20-cm and 30-cm depth there are still to be found many roots of the Dallis grass plants (about 30 per cent). Below this depth it is difficult to find any more roots, although a few roots may be found to a depth of 60 cm. The oxygen concentration data for this depth are presented in Figure 3. At this depth, the differences between the chlorotic and the green areas likewise remain throughout the sampling period, although the relationship between the dates of

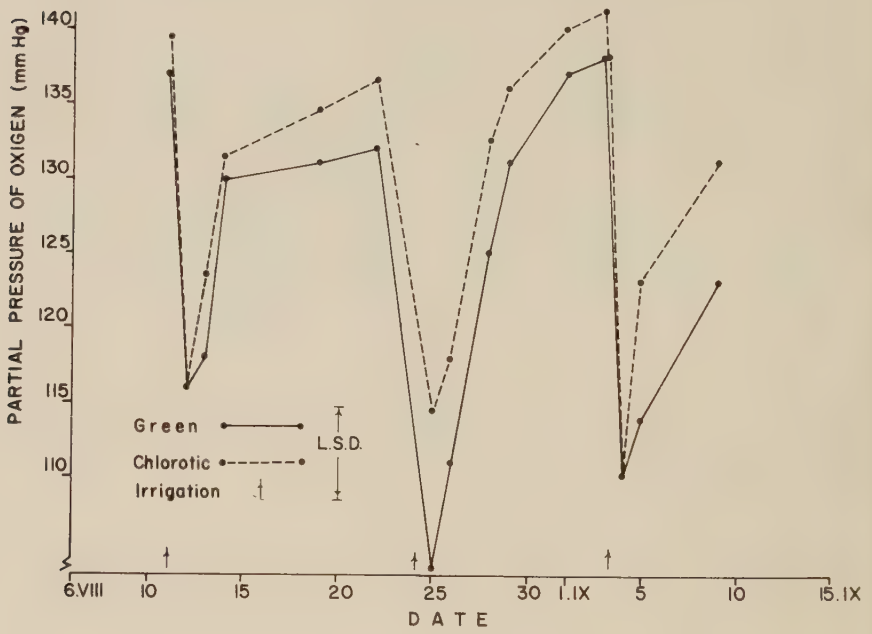

Fig. 2. Partial pressure of oxygen in the soil atmosphere at a depth of 30 cm.

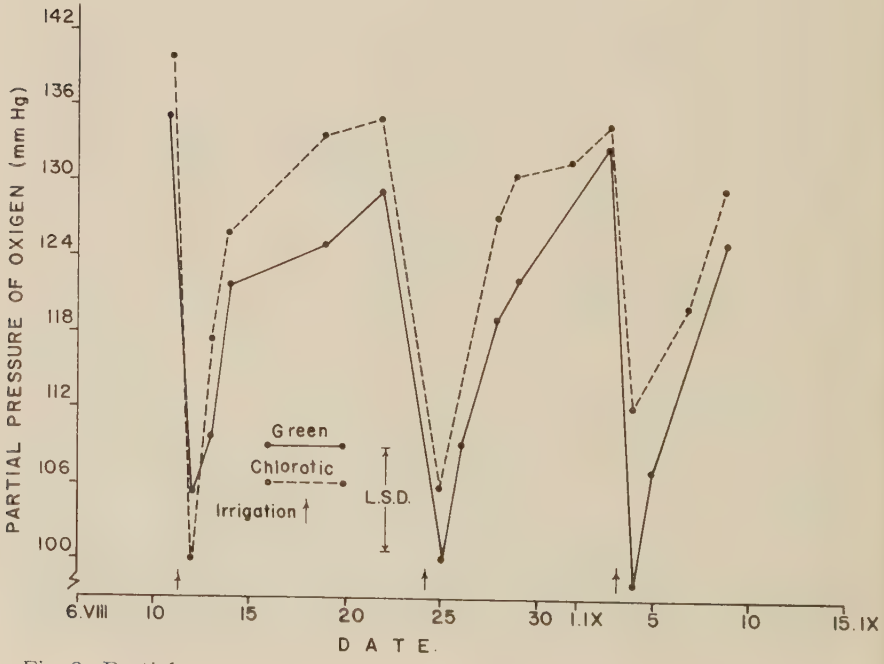

Fig. 3. Partial pressure of oxygen in the soil atmosphere at a depth of 60 cm.

significant differences and date of irrigation is not as clear as in the previous cases.

The results of the experiment carried out on the irrigated pasture at Yokneam show, therefore, that chlorosis is caused, or at least accompanied, by a high partial pressure of oxygen. In addition, it appears that a high oxygen concentration aggravates chlorosis only in excessively wet soils.

DISCUSSION

The purpose of the experiment described was to examine the influence of the partial pressure of oxygen in the soil atmosphere on the chlorophyll content of plant leaves. It is impossible to explain plant phenomena on the basis of the knowledge of oxygen concentration in the entire soil volume. In order to evaluate the role of this element in the physiology of the plant, it is necessary to know the conditions in the immediate vicinity of the plant root. The effectiveness of a method of measurement is to be judged on the basis of its ability to approach a true representation of the "effecfective" oxygen concentration (*i.e.* that found in the immediate vicinity of the root). The accepted methods are very limited in this respect, and thus the effectiveness of an oxygen determination is not so much a function of the method used, but of characteristics of the root system. The more a root system is branched, dense, and concentrated, the better are the chances that any measurement will represent the true situation.

In a measurement of oxygen concentration in the soil of a peach orchard at Har-El * and quince nursery at Mishmar Haemek ** (unpublished data), no differences were found between areas with green trees and those with chlorotic trees. Examination of the root systems showed that they were very sparse, so that the chances that the diffusion well would be near an active root were very small.

The irrigated pasture of Dallis grass and Rhodes grass represents the opposite situation to that of the peach orchard and the nursery. The pasture grasses have thick, dense, and well branched root systems, while their extent is limited. The soil is saturated with roots

* Jerusalem Hills;

** Western Jezreel Valley.

to a depth of 20 cm, and in lower horizons there is also a considerable number of roots. Such a situation is well adapted for evaluating the state of soil aeration. In addition, in such a situation the oxygen concentration reacts very rapidly to disturbances of the state of aeration. Such disturbances, caused by a temporary sealing of the soil by irrigation water, are clearly to be seen in Figures 1, 2, and 3. The oxygen consumption rate of the living root systems, their rhizosphere, and the micro-organisms which develop on the many plant remains, is very rapid, so that any inhibition of the oxygen supply results in an immediate and sharp decrease of the soil oxygen concentration. Under such conditions the direct measurement method of the partial pressure of oxygen has a good chance of being an accurate indicator of the soil aeration status, and although this method does not give the actual conditions prevailing immediately next to the roots, it is nevertheless possible to regard it as a reliable indicator of these conditions. From the results it appears that the large differences of oxygen concentration in the dense root zone (0 to 20 cm) between the area with green plants and that with chlorotic plants appear immediately after irrigation. The sharp decrease of oxygen concentration is similar in both areas, but the rate of recovery is different for the two areas. In the green area the recovery rate of the oxygen level is slower than in the chlorotic area.

From determinations of respiration rate of Dallis grass roots [7] it is evident that the roots of green plants absorb oxygen faster than those of chlorotic plants. It appears that the inhibition of rapid oxygen supply in the green area is caused, in addition to the physical properties of the soil, also by the very activity of the healthy roots. If we accept the hypothesis which suggests [3 5], that high partial pressure of oxygen is a cause of chlorosis, then it also appears that the plant has a mechanism of passive resistance acting against an increase of the partial pressure. When the plant becomes chlorotic for any reason, then this mechanism also breaks down, and the plant becomes even more vulnerable to damage by high partial pressure of oxygen.

Brown and Tiffin [2] discussed the effects of phosphorus and chelating agents, in the soil, upon chlorosis. It was found that compounds of iron with phosphorus and chelates are more stable when the iron is in its oxidized state. Brown and Tiffin [2] suggest that

the stable ferric compounds are less available to plant roots than compounds of iron in its reduced state. The reducing capacity of plant roots determines, for that reason, iron availability. It may be concluded, therefore, that oxygen interferes with the reduction of iron by roots, thus rendering it less available.

The discrepancy of the area "green B" from the general picture is the exception that proves the rule. The discrepancy mentioned confirms the idea [11] that soil moisture is an aggravating factor in chlorosis, and leads to the conclusion that it is not possible to predict plant behaviour concerning chlorosis without taking into account all the factors connected with this disorder.

It may be concluded that under field conditions the partial pressure of oxygen is a factor in the appearance of chlorosis. At the same time it must not be forgotten that a low oxygen concentration is generally accompanied by a relatively high level of CO_2 [9], and that the increase in CO_2-concentration also leads to an increase of the bicarbonate ion (in wet soil), and since this ion is apparently a principal, even if an indirect, cause of chlorosis [11], chlorosis should develop particularly on poorly aerated soils. This thesis is not confirmed by the facts observed. It appears that this inconsistency can be explained by the fact that the most active plant roots are found in the smaller, water-filled pores. Root respiration leads to an increase in the concentration of CO_2 in the soil atmosphere, and thus increasing its solubility and the concentration of the bicarbonate ion. It appears that these differences of concentration between the green and chlorotic areas are rather small, so that the level of CO_2 and bicarbonate causing chlorosis in equal or similar in both types of area, and that the chlorosis does not appear until the agravating factor of oxygen becomes operative.

SUMMARY

The oxygen concentration of the soil atmosphere was determined in an irrigated pasture sown to Dallis and Rhodes grass. It was found that variations in the chlorophyll content could not be explained on the basis of soil composition. A negative correlation between soil oxygen concentration and chlorophyll content of the leaves was found.

This correlation exists only at a certain moisture content. Changes of soil moisture have a strong influence on this relationship.

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THE ABSORPTION OF POTASSIUM AS INFLUENCED BY ITS CONCENTRATION IN THE NUTRIENT MEDIUM

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The amount of potassium (K) removed from the soil by plant roots is always much greater than the amount present in water-soluble form and often far exceeds the value obtained for exchangeable potassium.

Various theories have been proposed to explain the mechanism by which roots obtain cations from the soil. One of these, the soil solution theory, involves the absorption of K from the solution phase. Carbonic acid, formed by saturation of the soil solution with respiratory CO_2, reacts with soil particles releasing K-ions to the soil solution. If the K-ion concentration in the soil solution can be maintained above a deficiency level, plants growing in this soil will be able to produce good vegetative growth in the absence of deficiency symptoms. If the K-ion concentration is not maintained at an adequate level for plant growth some other mechanism such as that given by the contact theory [4] may be operating. This theory states that plant roots can obtain K-ions by a direct exchange of H-ion on the plant root for K-ion absorbed on the soil particle without the necessity of either ion entering the solution phase. The applicability of these two theories was examined by experiments with plants grown on Ramona loam soil.

The Ramona loam is a K-fixing soil containing 20,000 ppm of total K of which only 78 ppm is in the exchangeable form. When this soil was leached with CO_2-saturated water, 112 ppm of K were removed from the soil [11]. This value was considerably less than that released by rye seedlings in a Neubauer test (190 ppm). It was also found that, following the removal

of a quantity of K equal to that of the exchangeable K, the soil continued to release K at a rate of 0.1 mg per liter of leaching solution. This concentration of K, 0.1 ppm, was much lower (one-fifth) than had been found by previous investigators as a minimum for plant growth [1 2 5 6 7 8 9 10]. K-deficiency symptoms were found by Tyner [9] at a concentration of 0.5 ppm. His results were not confirmed by Bartholomew and Janssen [1], who found 0.5 ppm provided optimum growth for alfalfa and Hubam clover. Since the latter investigators worked with a solution volume 7.5 times that used by Tyner, it becomes evident that total supply as well as concentration is important. This fact is illustrated by an experiment of Johnston and Hoagland [5]. These workers obtained an increase in yield upon doubling the rate of flow of nutrient solution past the roots of tomato plants. They drew the conclusion that a K-concentration of 5 ppm would provide adequate growth for tomatoes.

Considering the work of these early investigators, it appeared unlikely that the low concentration of potassium (0.1 ppm) maintained in the Ramona loam would be adequate to prevent K-deficiency or to provide adequate growth.

EXPERIMENTAL

A solution-culture experiment was set up in the greenhouse at Berkeley, California. The apparatus (Fig. 1) consisted of 12 thirty-gallon galvanized iron cans fitted with plywood covers. The cans and the insides of the covers were painted with black asphaltum varnish. One hundred liters of one-fifth Hoagland-Arnon [3]. Solution minus-K was placed in each of the containers. The solutions were stirred and aerated by air passing through two glass tubes (E) which kept the solution in a rolling boil and by an air-driven percolator system (ABCF) which moved 100 liters of solution every $1\frac{1}{2}$ hours.

Two me of $Ca(NO_3)_2$, 0.8 me $MgSO_4$ and 0.2 me of $Ca(H_2PO_4)_2$ were added per liter of solution. In one experiment the concentrations of the above-listed macrosalts were decreased to one-tenth of the values listed. K was added to the solutions, as KNO_3 or KCl, at concentrations of 0.01, 0.1, 1.0, and 50 ppm. Micronutrients were added as recommended [3]. Distilled water and C.P. or Reagent-grade chemicals were used in preparing all solutions.

Atlas barley was used as the test plant. Barley seeds were germinated on a cheesecloth screen over distilled water. Seven days later, from 2 to 9 seedlings were transferred to each of the 12 tanks. Plants were harvested at 2-, 4-, and 6-week intervals, oven dried at 70°C, weighed, digested in HNO_3, and analyzed for K using a Beckman flame spectrophotometer. In one experiment the plants were grown in culture solutions for 11 weeks to determine the effect of seed-head production upon K-absorption. In this

experiment plants grown initially at a K-level of 0.1 ppm were transferred at 3 weeks to a 0.01-ppm tank. After 8 weeks of further growth the 0.01-ppm plants were compared with plants grown continually at 0.1, 1.0, and 50 ppm.

The K-concentration, pH, and temperature of the solutions were checked and adjusted twice daily. The concentrations of PO_4^{---} and NO_3^- in the solutions were checked every other day. K-analyses of the solutions were

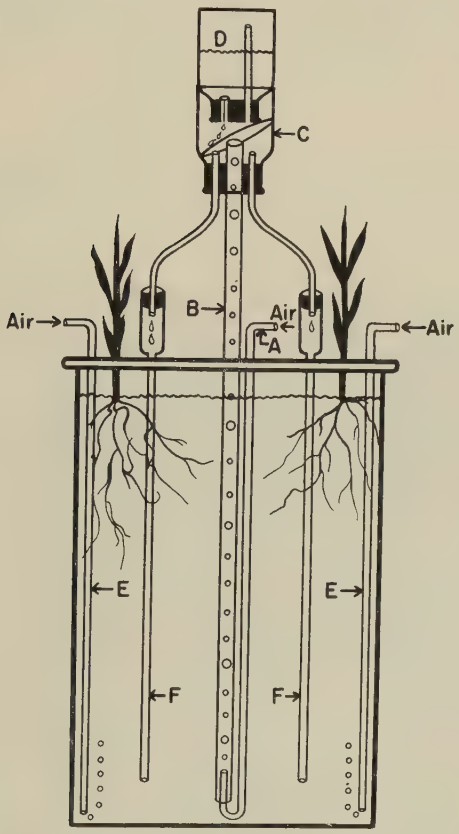

Fig. 1

made at 6 a.m. and 6 p.m. of each day after which the solution concentrations were adjusted to the assigned level. A dripping bottle D (Fig. 1) containing KNO_3 solution was placed on top of jar C. The concentration of potassium in D was adjusted each morning to meet the increased feeding ability of the plants. The K-solution dripped from a capillary tube into C at a rate of 80 ml in 24 hours where it was mixed with the percolating liquid, carried to the bottom of the tank in tubes F, and there mixed with the main

body of solution. Analyses of liquid taken from the top and bottom of the tank showed no variation in K content.

To examine the possibility of a competition between H^+ and K^+ the solution cultures were maintained at pH levels of 5.5 and 7.0. Potassium absorption was measured at these pH levels and at root temperatures of 15° and 25°C. In several experiments root temperatures were allowed to fluctuate with the temperature of the greenhouse. In the latter case, the overall temperature difference was between 16° and 24°C and depended upon the season of the year, weather conditions, and time of day.

RESULTS

Four experiments were designed and carried out to determine the yield and amount of K absorbed by barley plants in relation to the K concentration, macro-salt concentration, acidity, and temper-

Fig. 2

ature of the nutrient medium. The plants grew vigorously, tillered profusely, and by 11 weeks (Fig. 2) had produced many seed heads. Yields as a function of the K-content of the tops, roots, and seed heads are given in Figures 3, 4, and 5 respectively. A wide variation in both yield and K-content was noted.

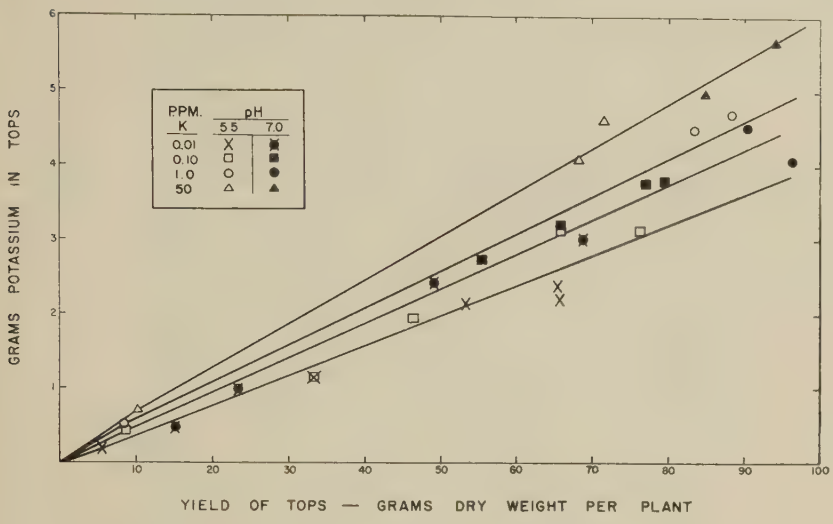

Fig. 3

Yield and K-content as a function of K-concentration

The lowest points shown on the curves of Figures 3 and 4 occurring at or below a yield of 10 grams, are the maximum yields of tops and roots obtained at 6 weeks growth. The lines drawn from the origin to these points include all data obtained in 3 experiments at 2-, 4-, and 6-week harvests. Each line represents data obtained from 20 to 112 plants. The remaining points on the graphs are for yields obtained at the 11 week harvest. It will be noticed that yield was extremely variable, with differences in growth observed between plants grown in the same or in duplicate tanks. The greatest variation in top growth observed for plants growing within the same tank was found at a K-concentration of 0.01 ppm (Table 1). In this tank, pH 7.0, top yields of 14.3 and 68.4 grams were obtained. A duplicate tank produced plants weighing 22.4 and 48.5 grams. Similar variations but of smaller magnitude were noted at all K-concentrations. The extreme tangling of roots which occurred in the tanks (Fig. 6) made it impossible to separate roots of different plants grown in the same tank, either for yield data or for plant analysis. However, variations in root yield were noted between duplicate tanks. All plants tillered profusely with the number of tillers varying from 15 to 81 per plant. Variations in

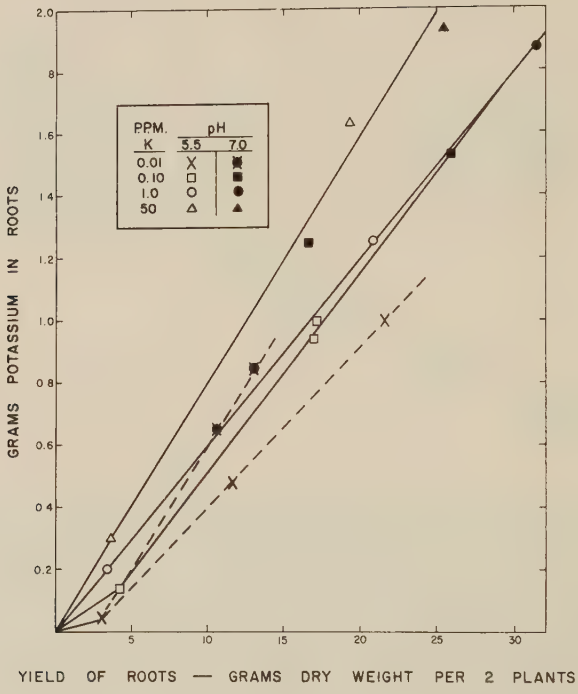
PPM. K
pH
5.5
7.0
0.01
0.10
1.0
50
GRAMS POTASSIUM IN ROOTS
YIELD OF ROOTS — GRAMS DRY WEIGHT PER 2 PLANTS

Fig. 4

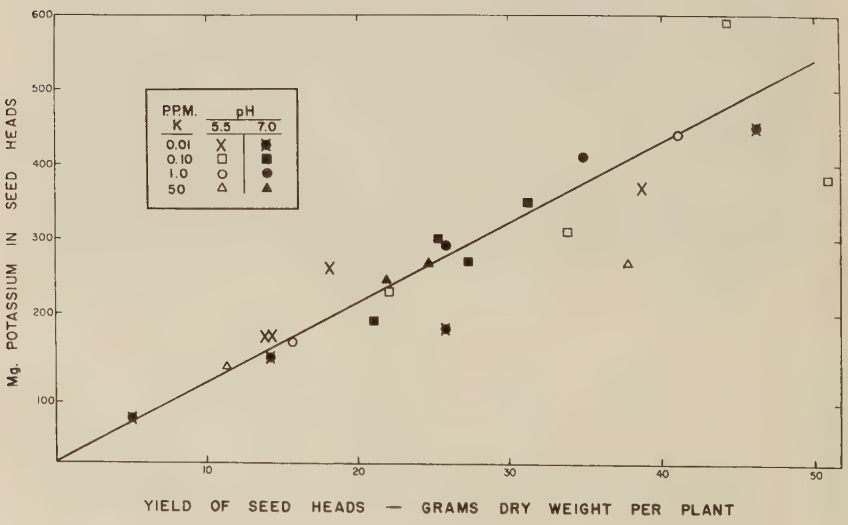
PPM. K
pH
5.5
7.0
0.01
0.10
1.0
50
Mg. POTASSIUM IN SEED HEADS
YIELD OF SEED HEADS — GRAMS DRY WEIGHT PER PLANT

Fig. 5

TABLE 1

Yield of barley (dry weight, grams per plant) at 11 weeks harvest								
Potassium concentration in tanks, ppm	pH 7.0				pH 5.5			
	Tops	Roots	Seeds	Number of tillers/plant	Tops	Roots	Seeds	Number of tillers/plant
0.01	48.5	10.6	14	39	52.6	11.7	17.9	46
	22.4		25.6	36	32.3		38.5	43
0.01	68.4	13	46.1	55	65.1	21.6	14.1	42
	14.3		4.8	15	64.8		13.6	42
0.1	54.5	16.5	20.8	42	32.6	17.1	33.6	47
	65.1		27.0	50	75.9		44.1	61
0.1	76.3	25.9	31	49	45.4	16.9	50.8	48
	79.0		25.1	50	65.2		21.8	42
1.0	96	31.4	34.6	50	87.9	20.8	40.9	57
	89.6		25.6	81	82.8		15.4	53
50	93.7	25.5	21.7	64	67.6	19.4	37.6	64
	84.6		24.4	53	70.8		11.1	47

top yield as well as seed head production (Figures 2 and 6, and Table 1) appeared to depend on the number of tillers a plant could produce and the time of production of the tillers, and not upon the K-concentration of the culture solution. The yield and K-content of the seed heads at the 11 week harvest are given in Figure 5.

The K-content of the tops increased with increasing concentration of K in the nutrient medium. Plants grown in a K-concentration of 50 ppm absorbed more K than plants grown at lower concentration. The plants grown at the lower concentrations, however, were able to produce greater growth per unit of K absorbed than were the plants grown at higher concentrations.

No potassium deficiency symptoms were observed at any of the K-concentrations studied.

The effect of temperature, pH, and macro-salt concentration on K absorption

Barley plants were grown for 6 weeks in solutions in which the temperature was maintained at 15 or 25°C, or allowed to vary with

Fig. 6

the temperature of the greenhouse (16 to 24°C). Acidities corresponding to pH values of 5.5 and 7.0 were maintained at each temperature. The temperature of the medium had only an indirect upon the amount of K absorbed by the plants. Plants grown at 25°C produced 50 to 70 per cent of the total dry matter produced by plants growing at 15°C, and absorbed a correspondingly smaller amount of K. In no instance did a difference in temperature cause a difference in the percentage K-content of the tops or roots.

Top, root, and seed yield for plants grown for 11 weeks were variable at both pH 5.5 and 7.0. At K-concentrations of 1.0 and 50.0 ppm, however, yield was markedly greater at pH 7.0 than at 5.5. No marked differences in the K-content of the tops were observed as a result of differences in the acidity of the solutions. However, at a K-concentration of 0.01 ppm, plant roots grown at pH 7.0 contained more K than roots grown in solutions at pH 5.5.

Decreasing the macro-salt concentration from 1/5 to 1/50 of the recommended Hoagland concentration had no appreciable effect on yield and no effect on the K-content of the plant.

K-concentration variability and absorption

The analytical values obtained for the K-concentrations in solution as measured in three of the tanks are shown graphically in Figure 7. Samples of the solutions were taken for analysis at

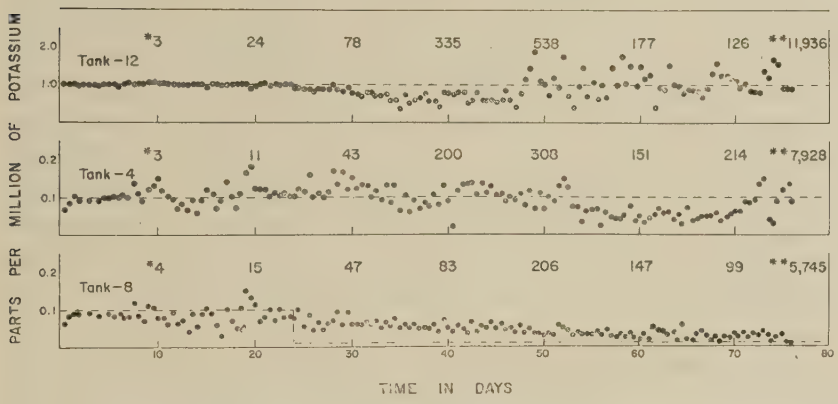

Fig. 7

6 a.m. and 6 p.m. each day for a period of 11 weeks. The concentrations of K at which the tanks were expected to be maintained are shown as dotted lines in the figure. Points lying above these lines indicate K-additions in excess of that required to maintain the concentrations at the appropriate levels. Points lying below the lines indicate K-additions insufficient to maintain the K-concentrations at the desired levels. It is to be noted that only occasionally did the value of K found in the tank coincide with the K-concentration expected.

Included in the graphs are the amounts of K absorbed by the plants on every 10th day and the total amount of K absorbed from the solutions at the end of the experiment. These values, identified by asterisks, are given at the top of each of the three graphs.

DISCUSSION

With the removal from the soil of a quantity of K equal to that found in the exchangeable form, the amount of K in the CO_2-saturated soil solution of the Ramona loam decreased from a relatively high concentration to a value of 0.1 ppm. Plants grown on this soil removed large quantities of non-exchangeable K. To do this they must have fed directly from the soil particles or have been able to feed adequately at the above 0.1 ppm concentration. From the work of previous investigators [1 5 6 7 8 10] using large-volume or flow-culture techniques, the latter mechanism would appear to be inadequate. These workers, with the exception of Bartholomew and Janssen, had apparently shown that K-concentrations of less than 0.5 ppm were inadequate for plant growth. Nevertheless, the possibility existed that plants growing in the soil at initially high K-concentrations might produce large root systems before the K-concentration in the solution phase dropped to the 0.1 ppm level. These plants might then more efficiently explore the soil solution and obtain sufficient K for adequate growth.

The experimental data presented in Table 1 and Figures 3 and 6 are from the 11-week experiment. This experiment was so arranged that plants grown in one set of tanks at a K-concentration of 1.0 ppm would be transferred at 4 weeks to solutions containing 0.1 ppm K. These plants, grown at 1.0 ppm, were expected to develop a much larger root system than those grown continuously in solutions maintained at 0.1 ppm and might, therefore, absorb more K and produce more plant material at 0.1 ppm than the 0.1 ppm control plants. At the end of a 3 week growth period it was clearly evident that little if any difference existed between plants grown in the 0.1 and 1.0 ppm K-concentrations. It was for this reason that the plants which had been grown at 0.1 ppm were transferred to solutions containing 0.01 ppm of K.

Figure 6 shows that the plants grown at 0.01 ppm of K are large, healthy plants and have produced a large amount of seed heads. Figure 3 shows the oven-dry weights of the shoots minus seed heads. Yield varied for plants in the same tank as well as for plants grown in different tanks. At a K-concentration of 0.01 ppm yield varied from 14.3 to 68.4 grams. Similar variation was noticed at

0.1 ppm with smaller variations at higher K-concentrations. Yield appeared to depend on the number of tillers which a plant could produce and had little if any correlation with the K-concentration of the culture solution.

The concentration of K present in a solution at any given instant depends upon the initial concentration, the rate of removal, and the rapidity with which the K can be replaced. In many of the previous experiments dealing with solution cultures a proper consideration of maintaining a constant concentration has undoubtedly been overlooked. A consideration of Figure 7 will illustrate this possibility. The figure shows the concentrations of K, in the nutrient solutions of 3 tanks, obtained twice daily from analyses extending over a period of 11 weeks. The dotted lines indicate the concentrations of K at which the tanks were expected to be maintained. As can be seen, this concentration was seldom found in the tanks, the difficulty being the problem of equalizing the supply and demand. To understand the enormity of the problem confronting anyone using a flow-culture technique at low concentrations, let us examine the data of Figure 7. On the 30th day of growth, 47 mg of K was removed from the 0.01 ppm tank by 2 barley plants. This value is equivalent to 4,700 liters of 0.01 ppm solution. If the plant is to decrease the concentration of the tank by only 10 per cent of the original concentration, then 47,000 liters must be flowed past these plants each day. On the 50th day the task becomes enormous with a volume of 206,000 liters required to pass the plant roots each day. If, as was done by some workers, only 2 liters of new solution were added or flowed past the plants per day, a deficiency might be observed even at a K-concentration of 0.5 ppm which adds only 1 mg of K to the plants each day. A total of 5.745 g of K was removed by the barley plants growing in Tank 8 (0.01 ppm K) over a period of 11 weeks.

In the first 3 experiments, which are not included in the figure, plants decreased the concentration of K in some tanks to values in the neighborhood of 0.001 ppm. This fact was also confirmed in an experiment with excised roots in KCl solution. 4.5 grams of roots (0.3 grams oven dried weight) were added to 3 liters of well aerated and stirred KCl solution at a concentration of 0.1 ppm. These plants removed all of the detectable K in a 3-hour absorption period at 26°C.

The above data suggests that plants growing on the Ramona loam can grow well on solution phase K as long as the concentration of K in the soil solution is maintained at or above a value of 0.02 to 0.03 ppm. The data, however, does not throw light on whether or not plants grown on the Ramona loam obtain significant amounts of K by a contact mechanism.

SUMMARY

Barley plants were grown, for periods of up to eleven weeks duration, in culture solutions in which the potassium concentrations were maintained at 0.01, 0.10, 1.0, and 50.0 ppm. Potassium added continuously during the day was augmented night and morning, as determined by solution analysis. The following observations were made:

Plants grew equally well at pH levels of 5.5 and 7.0. Variations in root temperature between 15° and 25°C had no effect on K-content of tissue at either pH level. Yields were large as were the quantities of potassium absorbed from the solutions. No potassium-deficiency symptoms were observed at any of the concentrations used.

Considerable variation in yield was observed between tanks and between plants growing in the same tank. The variation appeared to be due to a difference in the amount of tillering per plant.

The results indicate that plants grow well in culture solutions at potassium concentrations approaching 0.01 ppm and this may have some implications on the mechanism of uptake of potassium by plant roots growing in a soil system.

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